JOURNAL OF AGRICULTURAL RESEARCH

VOL. 65 WASHINGTON, D. C., AUGUST 1, 1942 No. 3

THE SEASONAL VERTICAL DISTRIBUTION OF WIREWORMS IN THE SOIL IN RELATION TO THEIR CONTROL IN THE PACIFIC NORTHWEST [1]

By E. W. JONES and F. H. SHIRCK, *assistant entomologists, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture* [2]

INTRODUCTION

Observations on the habits of wireworms (Elateridae) over a period of years have shown considerable variation in the amount of damage caused to crops by these pests in different months of the year. Samples of soil taken in connection with population studies have also indicated that these insects occur at various depths in the soil. The movement of wireworms in the soil has a decided bearing on the damage at different seasons, and consequently must be considered in any program of chemical or cultural control directed against them. A study of the vertical movement of wireworms in the soil was undertaken to determine more exactly their depth distribution through a complete season and under different conditions.

Attempts to trace the movements of individual wireworms having proved impractical, it was thought that the percentages occurring at different depths in short intervals of time would give a picture at least of the vertical movement in the soil. Observations were therefore made regularly in restricted areas of fairly heavy infestation, so that these movements could be studied in relation to soil temperature, moisture, and cropping conditions.

These observations were made near Walla Walla, Wash., and Parma, Idaho, during the period 1931–37.

REVIEW OF LITERATURE

In general, the published information regarding the depth distribution of wireworms is limited to fragmentary reports of casual observations. The economic importance of vertical migrations of wireworms in Australia has been pointed out by McDougall,[3] who states that the larvae of *Lacon variabilis*—

> are confined almost exclusively to badly drained country or parts of fields. The soil in most of these situations is from 9 to 14 inches in depth * * * and with an impervious clay subsoil. * * * The movement of larvae in the soil, according to moisture distribution, has resulted on occasions, in rather spectacular

[1] Received for publication September 25, 1941.

[2] The authors acknowledge their indebtedness to M. C. Lane, under whose supervision the work was done, for helpful suggestions and criticism of the manuscript. The portion of the investigation conducted at Parma, Idaho, was located on land furnished by the Idaho Agricultural Experiment Station under a cooperative agreement.

[3] McDOUGALL, W. A. THE WIREWORM PEST AND ITS CONTROL IN CENTRAL QUEENSLAND SUGAR-CANE FIELDS. Queensland Agr. Jour. 42: 690–726, illus. 1934.

effects. It has happened that some [sugarcane] fields known to be inhabited by a considerable *L. variabilis* larval population had been planted when the soil moisture had been very low. The results were good showings of primary shoots. At this stage a shower of rain was experienced. The larvae came up to the top 2 or 3 inches of moist soil and, as this moisture quickly disappeared, they descended. During this movement of the larvae they came into contact with primary shoots, etc., with the results that within three days following the shower fully 70 percent of the originally healthy primary shoots showed "dead-hearts."

A feature of special interest brought out in the study by McColloch, Hayes, and Bryson [4] in Kansas is that wireworms were frequently found above a depth of 6 inches in midwinter. In two seasons' observations the average depth at which wireworms were found from November through February ranged from 7.7 to 10.1 inches. These data were based upon the records of only a few individuals. In each digging the soil was removed inch by inch and examined by hand. The following conclusions of practical importance in wireworm control were drawn from these studies: (1) These insects can endure wide extremes of temperature, as is indicated by the shallow depth of hibernation, and (2) the often-recommended practice of fall plowing to expose them to the rigors of winter may have less value than is commonly believed.

Following an 8-year study at Manhattan, Kans., Bryson [5] presented a graphic summary of the number of wireworms taken in various habitats each month, together with the prevailing average soil temperatures. The data show that the larvae are below the plow line during November, December, and January and during July and the first half of August, but near the surface, or at least above the plow line, during the other 7 months. His observations were confined for the most part to three or four species of *Melanotus* and one species of *Aeolus*. As a result of this study Bryson suggests the desirability of seeking methods other than plowing for destroying the larvae.

METHODS

The soil-sampling technique was developed by workers of the Pacific Northwest wireworm project.[6] [7] Unit areas of 1 square foot of soil were dug in 3-inch layers to a depth of 18 or 24 inches and examined for wireworms at frequent intervals, usually once a week. The unit areas, ranging in number from 10 to 20, were dug at random over ⅛- to ¼-acre field plots. The soil of each layer was sifted through 16-mesh screens, and the wireworms were counted. For brevity the 3-inch layers below 12 inches are reported as one layer. The layers are designated as follows: Layer 1, 0 to 3 inches; layer 2, 3 to 6 inches; layer 3, 6 to 9 inches; layer 4, 9 to 12 inches; layer 5, 12 to 18 or 24 inches. No effort was made to obtain the smallest wireworms hatched from the current season's eggs, since other observations on

[4] McColloch, J. W., Hayes, Wm. P., and Bryson, H. R. Preliminary notes on the depth of hibernation of wireworms (elateridae, coleoptera). Jour. Econ. Ent. 20: 561-564. 1927.

[5] Bryson, Harry R. Observations on the seasonal activities of wireworms (elateridae). Kans. Ent. Soc. Jour. 8: 131-140, illus. 1935.

[6] Jones, E. W. Practical field methods of sampling soil for wireworms. Jour. Agr. Res. 54: 123-134, illus. 1937.

[7] Lane, M. C., and Shirck, F. H. A mobile power soil sifter. U. S. Bur. Ent. and Plant Quar. Cir. ET-70, 2 pp., illus. 1936. [Multigraphed.]

young wireworms have indicated that no significant variation in depth distribution from that of the older wireworms may be expected.

Soil-moisture determinations were made on the day of each soil sifting. Temperatures were obtained by means of soil thermographs stationed at Walla Walla on a standard fallow plot and on an alfalfa plot adjoining the field plots, and at Parma on a fallow plot.

The monthly mean depth of wireworms was calculated by multiplying the total number of wireworms found in each layer during the month by the mid-depth of the layer, adding the products for the various layers, and dividing by the total number of wireworms in all the layers.

The wireworms obtained at Walla Walla were predominantly *Limonius canus* Lec., but a few of the following species were also taken: *L. californicus* (Mann.), *L. infuscatus* Mots., and *L. subauratus* Lec. At Parma the predominating species was *L. californicus*, with about 10 percent of *L. canus*.

PRESENTATION OF DATA

WALLA WALLA, WASH.

The depth-distribution records obtained at Walla Walla are presented for four field plots as follows: In 1931 a sandy-loam spinach (*Spinacia oleracea* L.) field that was in crop during the spring and fall and fallow in the summer; in 1936 a silt-loam field that was uncropped during the year; and in 1937 a silt-loam 2-year-old alfalfa (*Medicago sativa* L.) plot and a silt-loam potato (*Solanum tuberosum* L.) plot. The alfalfa plot and the potato plot were irrigated during the crop season.

Spinach Plot

The data for the sandy-loam spinach field are presented in table 1.

It is shown that the spring upward migration began after soil at the 6-inch depth had warmed up to about 50° F. in March. The water content of the soil at that time was about 25 percent. The percentage of larvae in soil layers 1 and 2 increased during April, and by the last week of April the majority of the larvae were in these layers. But with the increase in soil temperature to 72° F. and the decrease in soil moisture of layer 1 to about 5 percent by the first of June, there was a tendency for the wireworms to move downward. An increase in the moisture content again following a June rain increased the percentage of wireworms in this layer for a short time only.

In July and August, when soil temperatures were at the seasonal peak and the soil moisture in layers 1 and 2 was decreasing gradually, there was a noticeable downward movement of the larvae, with a consequent increase in the percentage in layers 3 and 4, and there were also more larvae in layer 5 than in the late fall or early spring. During the first week of September, following the planting of the fall spinach crop and the late-summer irrigation, there was a slight upward movement of larvae to layer 1. The majority of the wireworms, however, remained below 6 inches during September and October.

TABLE 1.—*Seasonal vertical distribution of wireworms in a spinach plot at Walla Walla, Wash., in 1931 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]			
	1	2	3	4	5		Soil temperature at depth of 6 inches	Moisture in soil layer—		Rainfall
								1	2	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*°F.*	*Percent*	*Percent*	*Inches*
Mar. 9	0	6.9	34.6	44.6	13.8	130	48.2	24.9	22.3	0
Mar. 25	20.7	22.4	19.0	19.8	18.1	116	51.8	26.4	22.7	1.82
Average or total	9.8	14.2	27.2	32.9	15.8	246				
Apr. 6	43.2	27.2	14.8	2.5	12.3	81	50.0	22.9	24.3	1.87
Apr. 24	30.3	51.5	12.1	3.0	3.0	33	55.4	14.8	18.1	.12
Average or total	39.5	34.2	14.0	2.6	9.6	114				
May 9	19.6	50.0	19.6	2.2	8.7	46	64.0	13.7	15.9	.09
May 15	32.3	43.1	20.0	4.6	0	65	67.1	17.3	12.5	.24
Average or total	27.0	45.9	19.8	3.6	3.6	111				
June 4	12.7	46.8	32.9	7.6	0	79	71.6	4.5	11.6	0
June 12	13.0	47.7	29.9	9.3	0	107	70.7	2.7	17.0	.06
June 19	48.3	15.7	20.2	15.7	0	89	64.4	16.2	17.8	1.71
June 26	16.8	21.0	45.4	16.8	0	119	68.0	14.2	15.5	.05
Average or total	22.1	32.2	33.0	12.7	0	394				
July 3	15.2	24.1	42.1	18.6	0	145	73.8	5.7	13.8	.07
July 10	6.5	24.6	34.1	11.6	23.2	138	75.2	4.6	12.0	0
July 17	7.3	15.4	33.1	19.8	24.3	136	79.0	2.4	11.1	.14
July 25	6.9	10.3	20.7	16.4	45.7	116	80.6	4.3	11.3	0
Average or total	9.2	19.1	33.1	16.6	22.1	535				
Aug. 1	0	6.8	32.9	21.9	38.4	73	79.7	3.9	13.3	0
Aug. 10	1.1	4.3	34.0	38.3	22.3	94	77.0	2.1	11.2	0
Aug. 14	1.3	9.3	46.7	17.3	25.3	75	77.5	4.0	8.7	0
Aug. 22 [2]	4.5	22.7	18.2	9.1	45.4	22	67.4	18.0	18.6	.04
Average or total	1.1	8.0	38.0	25.4	29.5	264				
Sept. 5	20.0	20.0	26.7	0	33.3	15	77.5	10.1	15.0	0
Sept. 14	5.0	20.8	16.8	30.7	26.7	101	64.4	11.8	13.8	.11
Sept. 25	2.9	10.6	30.8	29.8	26.0	104	59.0	14.3	15.3	1.07
Average or total	5.0	15.9	24.1	28.2	26.8	220				
Oct. 3	8.1	9.7	33.9	27.4	21.0	62	60.4	7.9	10.1	0

[1] Average or total for week preceding date on which wireworms were counted.
[2] Plot irrigated Aug. 21.

FALLOW PLOT

The data for the fallow silt-loam field are presented in table 2.

Under bare-ground conditions the vertical movements of wireworms are closely related to soil temperatures. As soon as the soil temperatures began to rise above 40° F. in March, the wireworms moved upward into layers 1 and 2. After the middle of June soil temperatures exceeded 75° F., and the wireworms then moved downward. Unfavorable soil-moisture conditions, no doubt, were a factor in this downward migration. Thus, the highest percentages of wireworms in layer 1 were recorded in April and May, the highest in layer 2 occurred in May and June, in layer 3 in July and September, and in layer 4 in August.

TABLE 2.—*Seasonal vertical distribution of wireworms in a fallow plot at Walla Walla, Wash., in 1936 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]			
	1	2	3	4	5		Soil temperature at depth of 6 inches	Moisture in soil layer—		Rainfall
								1	2	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*°F.*	*Percent*	*Percent*	*Inches*
Mar. 12	0	2.6	52.6	36.8	7.9	38	45.0	25.2	25.7	0.15
Mar. 19	7.9	13.2	47.4	26.3	5.3	38	44.0	25.1	26.5	.02
Mar. 25	7.9	19.0	44.4	25.4	3.2	63	45.0	25.4	27.6	0
Average or total	5.8	12.9	47.5	28.8	5.0	139				
Apr. 1	12.2	17.1	34.1	29.3	7.3	41	41.0	25.4	27.6	.20
Apr. 13	40.0	20.0	13.3	20.0	6.7	15	57.0	16.4	23.1	.44
Apr. 17	18.5	48.1	11.1	18.5	3.7	27	66.0	16.4	23.1	0
Average or total	19.3	27.7	22.9	24.1	6.0	83				
May 1	60.0	26.7	6.7	6.7	0	15	63.0	6.8	20.2	.31
May 29	11.9	64.4	18.6	5.1	0	59	73.0	10.1	20.0	0
Average or total	21.6	56.8	16.2	5.4	0	74				
June 10	16.9	39.0	30.5	13.6	0	59	71.0			.39
June 22	10.9	19.6	43.5	15.2	10.9	46	77.5	4.6	12.2	.29
Average or total	14.3	30.5	36.2	14.3	4.8	105				
July 8	9.7	29.0	54.8	0	6.5	31	83.0	4.4	13.2	.02
July 20	5.3	5.3	57.9	5.3	26.3	19	85.0	2.3	6.6	.02
Average or total	8.0	20.0	56.0	2.0	14.0	50				
Aug. 1	2.6	28.9	21.1	36.8	10.5	38	80.5	2.3	9.3	0
Aug. 22	5.9	11.8	23.5	41.2	17.6	17	80.0	5.5	12.3	0
Average or total	3.6	23.6	21.8	38.2	12.7	55				
Sept. 29	0	18.7	58.8	13.8	8.8	80	66.0	6.3	9.2	0
Oct. 21	0	25.0	33.3	29.2	12.5	24	59.5			0

[1] Average or total for week preceding date on which wireworms were counted.

ALFALFA PLOT

The seasonal records for the silt-loam alfalfa plot are shown in table 3.

The weekly percentage of wireworms observed in layer 1 increased from 20 early in April to 54 by the middle of May. Early in the summer the percentage in layer 1 began to decline because of the heat and dryness of the soil. This downward migration was greatest after the alfalfa was cut on May 20, July 20, and August 23. As soon as a heavy rain occurred, like that of June 21, or following irrigations, more of the larvae were found in layer 1, and as the moisture rapidly decreased they descended. The wireworms in layer 1 ranged from about 54 percent in May to 6 percent in September. The percentages occurring in layers 2, 3, and 4 were more uniform and were lower than in layer 1.

TABLE 3.—*Seasonal vertical distribution of wireworms in an alfalfa plot at Walla Walla, Wash., in 1937 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]			
	1	2	3	4	5		Soil temperature at depth of 6 inches	Moisture in soil layer—		Rainfall [2]
								1	2	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*°F.*	*Percent*	*Percent*	*Inches*
Apr. 2	20.0	16.9	21.5	30.8	10.8	65	45.7	34.2	31.6	0.73
Apr. 9	46.0	20.6	19.0	7.9	6.3	63	46.9	29.8	27.5	.44
Apr. 16	47.0	15.7	19.6	9.8	7.8	51	50.2	31.1	30.1	1.10
Apr. 23	32.1	19.6	28.6	19.6	0	56	47.3	24.7	24.1	.22
Apr. 30	37.1	22.8	14.3	25.7	0	35	50.0	21.7	22.4	.26
Average or total	35.9	18.9	21.1	18.5	5.6	270				
May 7	48.8	22.0	9.8	14.6	4.9	41	52.5	12.3	15.8	0
May 14	34.9	34.9	18.6	7.0	4.6	43	53.6	18.4	16.2	.46
May 21	54.3	17.1	14.3	11.4	2.8	35	54.7	13.9	15.1	0
May 28	36.8	21.0	31.6	10.5	0	19	57.6	10.0	13.4	.02
Average or total	44.2	24.6	16.7	10.9	3.6	138				
June 4	25.0	50.0	20.0	5.0	0	20	62.2	6.1	13.8	0
June 11	26.9	30.8	26.9	15.4	0	26	65.3	14.3	13.5	.35
June 23	36.4	20.4	18.2	20.4	4.5	44	60.4	33.1	30.9	1.46
June 30	45.7	25.7	22.8	5.7	0	35	63.3	15.3	21.0	.06
Average or total	35.2	28.8	21.6	12.8	1.6	125				
July 7	23.5	27.4	31.4	13.7	3.9	51	70.3	15.2	16.0	0
July 16	36.4	21.2	21.2	18.2	3.0	33	70.7	11.7	14.1	0
July 23	17.2	27.6	37.9	13.8	3.4	29	76.1	9.2	13.8	0
July 30	29.4	29.4	35.3	5.9	0	17	70.1	27.1	28.1	.18
Average or total	26.2	26.2	30.8	13.8	3.1	130				
Aug. 6	36.8	42.1	13.2	7.9	0	38	70.9	21.1	21.0	0
Aug. 12	32.2	16.1	22.6	22.6	6.4	31	68.7	16.7	18.2	0
Aug. 20	33.3	26.7	28.9	8.9	2.2	45	66.2	15.8	16.4	.15
Aug. 26	26.5	17.6	29.4	26.5	0	34	64.0	12.6	15.0	.04
Average or total	32.4	26.4	23.6	15.5	2.0	148				
Sept. 3	36.4	13.6	22.7	27.3	0	22	64.8	16.9	17.6	.06
Sept. 9	31.7	29.3	29.3	7.3	2.4	41	62.6	24.2	24.8	.20
Sept. 16	6.1	26.5	40.8	24.5	2.0	49	61.7	21.2	22.0	0
Sept. 23	13.0	19.5	37.0	23.9	6.5	46	58.3	20.6	20.2	0
Sept. 29	18.0	28.0	32.0	20.0	2.0	50	55.6	21.7	21.7	0
Average or total	18.8	24.5	33.6	20.2	2.9	208				
Oct. 6	17.6	23.5	23.5	27.4	7.8	51	55.6	23.6	22.8	.34
Oct. 13	12.0	16.0	40.0	28.0	4.0	50	53.8	23.6	22.5	0
Oct. 20	21.6	21.6	35.1	21.6	0	37	49.8	23.4	22.1	.23
Oct. 27	18.8	18.8	15.6	28.1	18.8	32	52.9	22.5	22.0	0
Average or total	17.0	20.0	29.4	26.5	7.1	170				

[1] Average or total for week preceding date on which wireworms were counted.
[2] Plot irrigated on June 7, July 13 and 25, Aug. 30, and Sept. 4 and 11.

POTATO PLOT

The seasonal records for the silt-loam potato plot are shown in table 4. In this plot the soil was dug between the rows.

Fairly uniform numbers of wireworms between 20 and 40 percent were present in layer 1 in April and May. The percentage in this layer went below 20 in June and July owing to heat and dryness, but after the heavy rains late in June the percentage increased, and as the moisture disappeared with rising temperatures the larvae descended. The same response was noted following irrigations throughout the

summer. In general, the percentages in layer 2 were higher than in layers 1 and 3 throughout the spring and summer. In layer 3 the percentages were low in the spring and high in the summer and fall. In layer 4 they were low in the spring and early summer, rose in August, and remained high through October. Few larvae remained below 12 inches from May to August, inclusive, and about as many were below this depth in April as in September.

In spite of the greater accumulation of wireworms in the soil of a potato hill as compared with that in soil between rows, essentially the same depth distribution as reported has been observed.

TABLE 4.—*Seasonal vertical distribution of wireworms in a potato plot at Walla Walla, Wash., in 1937 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]			
	1	2	3	4	5		Soil temperature at depth of 6 inches	Moisture in soil layer—		Rainfall [2]
								1	2	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*°F.*	*Percent*	*Percent*	*Inches*
Apr. 6	21.3	21.3	27.6	25.5	4.2	47	48.0	31.5	31.5	1.11
Apr. 12	21.3	36.2	10.6	19.1	12.8	47	51.0	28.1	27.2	.27
Apr. 19	14.3	50.0	10.7	10.7	14.3	28	51.0	25.4	27.9	.89
Apr. 26	22.2	55.6	13.9	5.6	2.8	36	53.5	21.1	22.7	.22
Average or total	20.2	38.6	16.4	16.4	8.2	158				
May 3	22.6	58.1	19.4	0	0	31	60.5	17.6	22.3	.26
May 10	28.6	45.7	14.3	5.7	5.7	35	61.0	13.4	18.6	.18
May 17	40.4	50.0	9.6	0	0	52	64.0	17.9	21.1	.28
May 24	29.5	61.4	9.1	0	0	44	68.0	11.1	19.8	0
Average or total	31.5	53.7	12.3	1.2	1.2	162				
June 2	32.4	29.4	29.4	8.8	0	34	70.0	10.5	19.0	.02
June 9	17.1	58.5	17.1	7.3	0	41	73.0	12.2	14.0	.04
June 14	13.3	30.0	46.7	10.0	0	30	71.0	23.3	26.6	.31
June 23	17.8	37.8	35.6	4.4	4.4	45	68.0	30.0	27.3	1.46
June 30	46.9	15.6	25.0	12.5	0	32	78.5	11.6	20.0	.06
Average or total	24.7	35.7	30.2	8.2	1.1	182				
July 9	7.1	46.4	46.4	0	0	28	80.0	18.4	23.8	0
July 14	22.2	40.7	33.3	3.7	0	27	84.0	14.1	22.1	0
July 21	11.8	61.8	23.5	2.9	0	34	84.0	19.4	22.7	0
July 29	0	44.0	40.0	16.0	0	25	86.0	21.3	23.9	.18
Average or total	10.5	49.1	35.1	5.3	0	114				
Aug. 4	20.0	53.3	26.7	0	0	15	77.0	31.5	32.2	0
Aug. 10	19.0	42.8	23.8	14.3	0	21	79.0	19.9	22.8	0
Aug. 17	26.3	26.3	26.3	15.8	5.3	19	76.5	15.5	20.4	.15
Aug. 26	0	27.8	44.4	27.8	0	18	76.0	24.3	25.5	.04
Average or total	16.4	37.0	30.1	15.1	1.4	73				
Sept. 1	0	18.4	47.4	26.3	7.9	38	71.0	21.9	19.5	.06
Sept. 8	6.7	20.0	40.0	33.3	0	15	71.0	25.6	25.9	.20
Sept. 21	3.3	26.7	33.3	30.0	6.7	30	70.0	28.5	28.1	0
Sept. 29	14.3	7.1	35.7	28.6	14.3	14	63.0	23.3	25.5	0
Average or total	4.1	19.6	40.2	28.9	7.2	97				
Oct. 6	12.5	25.0	43.8	18.8	0	16	57.5	26.8	27.2	.34
Oct. 13	4.2	25.0	37.5	33.3	0	24	57.0	25.8	25.3	0
Oct. 20	3.0	12.1	39.4	33.3	12.1	33	56.0	23.9	24.4	.23
Oct. 27	0	16.1	38.7	35.5	9.7	31	57.0	21.1	22.6	0
Average or total	4.8	18.3	39.4	31.7	6.7	104				

[1] Average or total for week preceding date on which wireworms were counted.
[2] Plot irrigated June 7, July 6, and August 6 and 18.

PARMA, IDAHO

The observations at Parma were made during the growing seasons of 1936 and 1937. In 1936 the studies were conducted in one plot only, in which potatoes were grown; in 1937 a fallow plot and a corn plot were used. Each plot was one-sixth of an acre, and all the plots were located on the same 5-acre tract, where the soil was of a clay-loam type. The fallow plot was not irrigated, but the corn and potato plots were irrigated several times during the growing season.

POTATO PLOT

The data for the potato plot are presented in table 5. The first examination was made on March 16, nearly a month before the

TABLE 5.—*Seasonal vertical distribution of wireworms in a potato plot at Parma, Idaho, in 1936 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]	
	1	2	3	4	5		Soil temperature at depth of 6 inches	Rainfall [2]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*° F.*	*Inches*
Mar. 16	4.2	16.8	34.8	25.1	18.8	95	41.9	0.06
Apr. 16	30.8	9.6	11.8	22.8	25.0	136	56.1	0
May 8	40.0	44.0	8.0	4.0	4.0	25	63.3	.12
May 15	53.2	40.6	3.2	3.2	0	32	65.8	.02
May 23	42.4	24.2	18.2	9.1	6.0	33	73.9	0
Average or total	45.2	36.3	9.8	5.4	3.3	90		
June 5	42.2	26.4	26.4	0	5.2	19	74.8	1.26
June 18	30.8	18.0	20.6	23.0	7.8	39	75.9	.03
June 27	3.6	35.8	57.2	3.6	0	28	77.5	.18
Average or total	25.6	26.8	34.7	8.8	4.0	86		
July 3	13.8	41.4	31.0	6.8	6.8	29	81.0	0
July 10	14.2	8.6	60.0	11.4	5.6	35	82.9	.14
July 18	5.6	40.8	24.0	22.2	7.6	54	78.1	.02
July 25	22.2	26.0	26.0	14.8	11.2	54	85.5	0
July 30	5.8	12.8	43.8	20.6	16.4	102	86.2	0
Average or total	12.4	26.0	36.8	15.2	9.6	274		
Aug. 7	10.6	19.2	38.6	19.2	12.2	57	85.3	.29
Aug. 14	5.2	14.0	21.0	33.4	26.6	57	84.2	.02
Aug. 21	2.8	17.4	49.2	20.2	10.0	69	82.9	0
Aug. 26	5.2	30.8	30.7	23.0	10.2	39	81.5	0
Average or total	6.0	20.4	34.9	24.0	14.8	222		
Sept. 2	2.8	31.4	38.6	17.2	10.0	70	77.7	.69
Sept. 9	4.2	22.8	37.2	21.4	14.4	70	71.2	.25
Sept. 24	0	14.6	50.0	18.8	16.8	48	64.9	0
Average or total	2.4	23.0	42.0	19.2	13.8	188		
Oct. 13	3.6	32.2	19.6	14.2	30.2	56	62.8	0
Oct. 30	1.4	30.4	13.0	26.0	29.0	69	51.6	0
Average or total	2.5	31.3	16.3	20.1	29.6	125		

[1] Average or total for week preceding date on which wireworms were counted.
[2] Plot irrigated May 29, June 27, July 3, 9, and 21, and Aug. 30.

potatoes were planted. At this date only 4 percent of the wireworms were in layer 1, which showed that little upward movement had yet occurred. The average soil temperature for the preceding week was

about 42° F. At the next observation, on April 16, the wireworms in layer 1 had increased to 31 percent of the total, and the percentage in this layer was consistently above 30 until after June 18. The seasonal peak, 53 percent, was reached about May 15 and was preceded by an average temperature of 65.8° F. After June 18 the wireworms began to leave the surface zone, and most of them stayed below the 3-inch depth the remainder of the season. Layer 3 contained the highest percentage of wireworms from June to September.

The potatoes, which were planted on April 10, did not come up until about May 10, and did not provide much shade before May 20. By August 20 the vines were beginning to dry up, affording less shade from then on until the potatoes were harvested. The principal effects of irrigation were noted in layer 1, where wireworms that had been driven down by heat and the dryness of the soil tended to return and remain near the surface for a time following irrigations in June and July.

Corn Plot

Soil observations were made as early as April 27, but the corn was not planted until June 9 and it was not large enough to afford much shade until the end of June.

The data for this plot are presented in table 6. From observations made in a neighboring plot, it is known that the wireworms became

Table 6.—*Seasonal vertical distribution of wireworms in a corn plot at Parma, Idaho, in 1937 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]	
	1	2	3	4	5		Soil temperature at depth of 6 inches	Rainfall [2]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*° F.*	*Inches*
Apr. 27	28.8	11.2	17.8	26.6	15.4	45	55.0	0.12
May 21	61.6	27.0	3.8	7.6	0	26	68.7	.94
June 10	20.0	37.0	35.0	2.6	5.2	40	70.0	.16
June 17	53.4	25.8	19.0	0	1.8	58	66.0	.09
June 24	21.4	42.8	34.0	1.8	0	56	68.2	.08
Average or total	31.6	35.2	29.3	1.5	2.3	154		
July 7	0	38.0	47.6	12.0	2.4	42	79.7	0
July 15	8.8	29.4	32.4	17.6	11.8	34	81.0	.12
July 22	5.6	37.0	46.2	7.4	3.8	54	82.6	0
July 29	7.8	63.6	27.2	0	1.2	77	84.0	.09
Average or total	5.6	42.0	38.5	9.3	4.8	207		
Aug. 5	11.4	38.6	45.4	2.2	2.2	44	78.4	.09
Aug. 12	0	29.0	45.2	22.6	3.2	31	84.0	0
Aug. 19	5.4	45.4	21.8	7.2	20.0	55	82.6	0
Aug. 26	13.8	20.6	20.6	31.0	13.8	29	80.4	0
Average or total	7.7	33.4	33.3	15.8	9.8	159		
Sept. 2	10.0	45.0	27.6	10.0	7.6	40	75.0	0
Sept. 9	36.2	13.8	19.4	11.2	19.4	36	72.7	.01
Sept. 15	0	40.0	30.0	10.0	20.0	30	74.1	0
Sept. 24	2.0	14.0	20.0	32.0	32.0	50	70.0	.50
Sept. 30	1.6	6.6	34.4	29.6	28.0	61	62.6	0
Average or total	10.0	23.9	26.3	18.5	21.4	217		

[1] Average or total for week preceding date on which wireworms were counted.
[2] Plot irrigated June 3 and 12, July 6 and 22, and Sept. 2.

active and began to move upward about the first of April. On April 27, 29 percent were found in layer 1. A rainfall of 0.94 inch on May 19 was followed by an accelerated movement of wireworms into layers 1 and 2, and the population on these layers reached 89 percent on May 21. Although there was no immediate increase in soil temperature, the percentage of wireworms in these layers had dropped to 57 by June 10. One week later, however, the percentage above 6 inches had again increased to 79. This upward movement was probably a response to the drop in soil temperature and to the rainfall and the irrigation between June 10 and 17. The presence of abundant food in the form of germinating corn also doubtless played a part. Wireworms were found congregated in the corn rows feeding on the sprouting corn on June 17.

A strong downward movement began toward the end of June, and on July 7 no wireworms were found in layer 1. The population of layer 2 also showed a downward trend from June 24 until July 15. During this period of the summer the mean weekly temperature rose from 68.2° to 81° F.

The increasing shade afforded by the growing corn, together with rains and the irrigations of July 6 and 22, brought about favorable conditions in the upper layers, which were reflected in the accumulation of wireworms in layer 2 and a slight increase in layer 1 during the latter part of July. Layer 3 showed no definite trend during July, but layer 4 showed a small accumulation, reaching a peak of 17.6 percent on July 15. From July to the end of September the percentage of wireworms in this layer was irregular. The 30 percent found here at the end of the season was probably about the percentage that passed the winter at this level. An accumulation of wireworms in layer 5 began about the middle of August.

In layers 2 and 3 wireworms were found at every observation throughout the season. Layer 2, containing the highest maximum and the highest minimum for the season, and the highest monthly average percentage of wireworms, was on the whole the most heavily populated layer during the season. Layer 1 showed the greatest variation during the season, the percentage ranging from 62 on May 21 to 0 on July 7, August 12, and September 15.

Fallow Plot

In the fallow plot no weeds were permitted to grow and no irrigations were made.

The data on the wireworm distribution are presented in table 7. Although the wireworms tended to remain deeper in the soil throughout the season than in either the corn plot or the potato plot, the seasonal movement was closely correlated with the trends observed in the other two plots.

A rapid movement of wireworms to the surface began about the first of April, and during April and May the largest percentages were found in layer 1. On May 27, 64 percent were found here, but by June 3 the percentage had dropped to 24. Most of the wireworms did not immediately go deeper than 6 inches, for on the same date layer 2 contained 52 percent of them, an increase of 42 percent over the

TABLE 7.—*Seasonal vertical distribution of wireworms in a fallow plot at Parma, Idaho, in 1937 in relation to soil temperature and moisture*

Date	Wireworms found in soil layer—					Total wireworms	Weather data [1]			
	1	2	3	4	5		Soil temperature at depth of 6 inches	Moisture in soil layer—		Rainfall
								1	2	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*°F.*	*Percent*	*Percent*	*Inches*
Apr. 2	11.6	11.6	8.4	25.0	43.2	60	48.2	21.7	20.6	0.38
Apr. 9	35.4	10.4	20.8	12.4	20.8	48	45.9			.23
Apr. 16	32.6	19.2	19.2	15.4	13.4	52	52.0			0
Apr. 23	53.0	17.6	11.8	7.8	9.8	51	53.6			0
Average or total	33.0	14.7	15.0	15.2	21.8	211				
May 6	43.6	25.6	12.8	2.6	15.2	39	62.1	13.2	16.5	.12
May 13	32.6	20.0	10.0	15.0	22.4	40	64.9			.05
May 21	54.6	13.6	9.0	16.0	6.8	44	68.7			.94
May 27	64.2	10.2	7.6	12.8	5.0	39	71.4	7.9	15.5	.02
Average or total	48.6	17.4	9.9	11.6	12.4	162				
June 3	24.2	51.8	17.2	3.4	3.4	29	67.5			0
June 9	7.6	15.4	46.2	11.6	19.2	26	70.0			.12
June 17	21.2	18.2	39.4	12.0	9.0	33	66.0	14.7	18.2	.13
June 24	15.4	25.0	27.0	17.4	15.4	52	68.2			.08
Average or total	17.1	27.6	32.5	11.1	11.8	140				
July 1	0	32.2	48.4	3.2	16.0	31	73.8			0
July 7	0	19.6	17.0	19.6	44.0	41	79.7	17.0	22.9	0
July 15	19.0	13.6	32.4	16.2	19.0	37	81.0	10.5	17.0	.12
July 21	3.0	26.2	29.2	13.8	27.6	65	82.6			0
July 29	0	29.8	23.4	23.4	23.4	47	84.0	8.4	16.7	.09
Average or total	4.0	24.4	30.1	15.2	26.0	221				
Aug. 5	7.6	30.8	27.0	11.6	23.2	26	78.4			.09
Aug. 12	0	14.0	28.0	17.2	40.6	64	84.0	12.2	18.5	0
Aug. 19	5.8	30.8	21.2	15.4	26.8	52	82.6	12.1	11.1	0
Aug. 26	0	13.6	31.8	31.8	22.6	44	80.4			0
Average or total	3.4	22.3	27.0	19.0	28.3	186				
Sept. 2	1.4	32.0	34.6	14.6	17.4	75	75.0	8.8	18.3	0
Sept. 9	2.4	19.6	39.0	31.8	7.4	41	72.7	8.2	12.1	.01
Sept. 15	4.4	22.2	20.0	17.8	35.8	45	74.1	9.0	14.3	0
Sept. 24	0	7.4	19.6	19.6	53.8	41	70.0	11.6	17.0	.50
Average or total	2.1	20.3	28.3	21.0	28.6	202				

[1] Average or total for week preceding date on which wireworm counts were made.

population found at this level 1 week earlier. On June 9, 46 percent were found in layer 3, this being 29 percent more than were found in this layer the previous week, and comparison with layers 1 and 2 will reveal that this increase was occasioned by downward penetration of wireworms from above 6 inches.

After the first of June wireworms were in general progressively less numerous in layer 1, although they tended to return for a brief time following the occasional showers that occurred in June, July, and August. Showers in the third week of September, however, caused no upward movement, for by that time the surface-soil temperature had fallen below those favored by wireworms, and they were attracted to the warmer soil below.

COMPARISON OF VERTICAL DISTRIBUTION OF WIREWORMS IN FIELD PLOTS AT WALLA WALLA, WASH.

The cumulative percentages of wireworms for successive layers of soil by months and plots at Walla Walla are presented in figure 1.

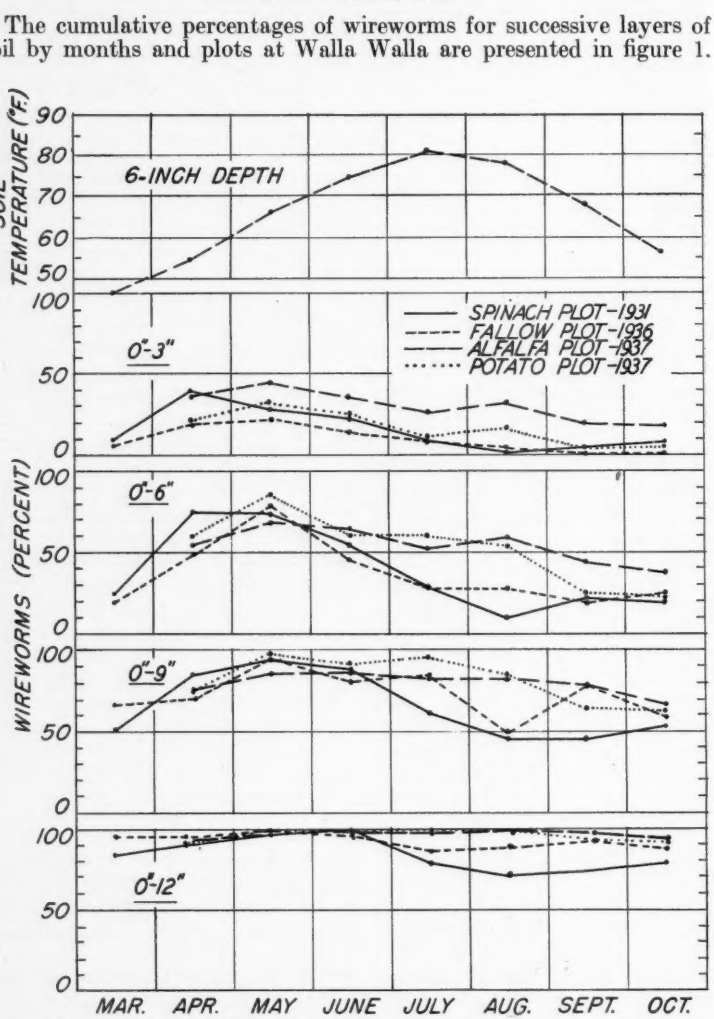

FIGURE 1.—Soil temperature at 6-inch depth and monthly percentages of wireworms in different soil layers at Wall Walla, Wash.

The maximum percentage of wireworms within 3 inches of the soil surface occurred during April and May, when there were from 19 to 44 percent in this layer of soil. During July and August high soil temperatures and low soil moisture tended to drive the wireworms downward to greater depths. However, in the potato and alfalfa plots the

percentage remaining in the upper 3 inches was greater than in fallow plots.

From 47 to 85 percent of the wireworms were in the first 6 inches of soil during April and May. In the fallow and spinach plots, with exposed soil surfaces, the percentages dropped below 30 in July, but in the alfalfa and potato plots more wireworms, up to 59 percent, were found above the 6-inch depth.

The percentage of larvae within 9 inches of the soil surface was from 86 to 95 during May but decreased in the fallow and spinach plots during June and July so that in these plots it was less than 50 during August.

Practically all the larvae in all the plots were in the first four layers of soil during May and June, and in the potato and alfalfa plots there were never less than 90 percent in the upper 12 inches. In the fallow plot, however, only 86 percent were found in these layers in July, and in the spinach plot only 70 percent were found there in August.

At Parma, Idaho

The cumulative percentages of wireworms for successive layers of soil by months and plots at Parma are presented in figure 2. The potato plot was planted April 10, but no important amount of ground cover developed before the middle of May. The corn plot was not planted until June 9. Hence all three plots at Parma were essentially devoid of ground cover during March and April, and no significant divergence in wireworm movements was expected. It did seem surprising that the close agreement in seasonal trend continued for the three cover situations during the remainder of the summer. From May to September the percentages of wireworms in all layers were lower in the fallow plot than in either of the two cropped plots. This was to be expected, since in the fallow plot there was little food to detain them at the higher levels, and, moreover, there were greater extremes of heat and dryness, factors that would cause them to remain deep in the soil.

The percentage of wireworms within 3 inches of the soil surface never exceeded 62, and that percentage was approached in May only. In July they practically disappeared from this layer. The percentage of larvae within 6 inches of the soil surface reached a maximum of 88 during May and dropped below 50 in each plot during July. The percentage of wireworms within 9 inches of the soil surface reached 96 in the corn plot during June. Below the 12-inch depth of soil the average percentages present in May and June ranged from 12 for the fallow plot to 0 for the corn plot. There was a gradual downward migration during the summer months, and in August about 28 percent of the wireworms that were found in the fallow plot were more than 12 inches below the soil surface.

MONTHLY MEAN DEPTH OF WIREWORMS

The monthly mean depths throughout the season at Walla Walla (fig. 3) show that the wireworms move toward the surface in all plots very early in the spring. This migration began early in March, when the temperature of the soil was 40° F. or above. The upward movement ceased after April in the spinach plot and after May in

the other plots. The average soil temperature when the average depth of the wireworms was 4 to 5 inches was approximately 66° F.

Coincident with changes in the soil environment at the surface, especially when the average soil temperature reached 75° F., there

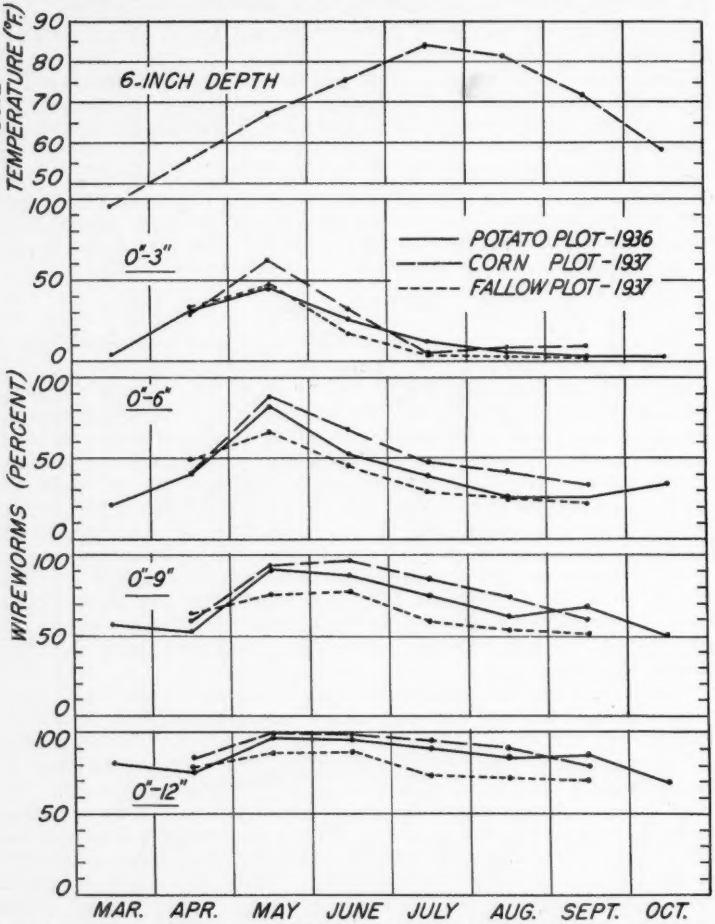

FIGURE 2.—Soil temperature at 6-inch depth and monthly percentages of wireworms in different soil layers at Parma, Idaho.

was a downward movement which continued into July in the alfalfa plot and into August in the other plots. The exposed soils of the spinach and fallow plots show the greatest vertical movement, the average depths in August being 10.5 and 8.5 inches, but in the shaded alfalfa and potato plots the average depths were 5.5 and 6 inches. A slight upward movement occurred in the fallow and spinach plots during September, when soil-surface conditions again became favorable for

wireworms. At this season the average temperature was again near 70° F., and moisture from irrigations and fall rain had wet the surface soil. On the other hand, the wireworms in the alfalfa and potato plots continued their downward movement during September and October, following the drop in temperature. Here it appears that wireworms definitely prefer the warmer subsoil temperatures. At the close of the season the wireworms in the alfalfa plot were at an average depth of 7 inches, in the potato and fallow plots 8.5 inches, and in the spinach plot 9.5 inches.

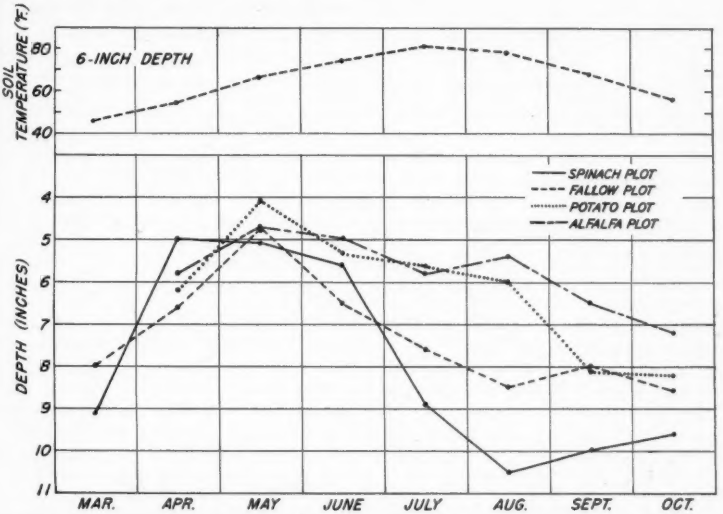

FIGURE 3.—Monthly mean depth of wireworms in the first 18 inches of soil, with monthly mean soil temperatures for the surface 6 inches (7-year average) at Walla Walla, Wash.

At Parma (fig. 4), although the studies were carried on in both fallow and cropped situations, the general trend in depth distribution was closely parallel in the three plots studied. The greatest seasonal range in vertical movement occurred in the cropped plots, being 5.1 inches for both the corn and the potato plot, and 4.1 inches for the fallow plot. The seasonal movement downward after May was more abrupt and consistent in all plots than at Walla Walla. Although there is a suggestion of an upward movement in September in the fallow and potato plots, no such incipient trend is shown for the corn plot. Apparently, the secondary upward movement of wireworms described by Bryson [8] as occurring in September in Kansas is not characteristic of the behavior of these insects in the Parma region.

DISCUSSION

Notwithstanding differences in soil type as between Walla Walla and Parma, and in spite of any conceivable influences imposed by

[8] See footnote 5.

variations in climate, or diversity of crops grown in the experimental plots, certain points of similarity in seasonal movements of wireworms are demonstrated for the two localities. The majority of the larvae passed the winter below the 6-inch depth. A study of several years' notes of observations in the early spring showed that an upward movement began about the first of March, or when the mean weekly soil temperature at 6 inches reached 40° F., and this movement proceeded gradually for several weeks, corresponding closely with seasonal

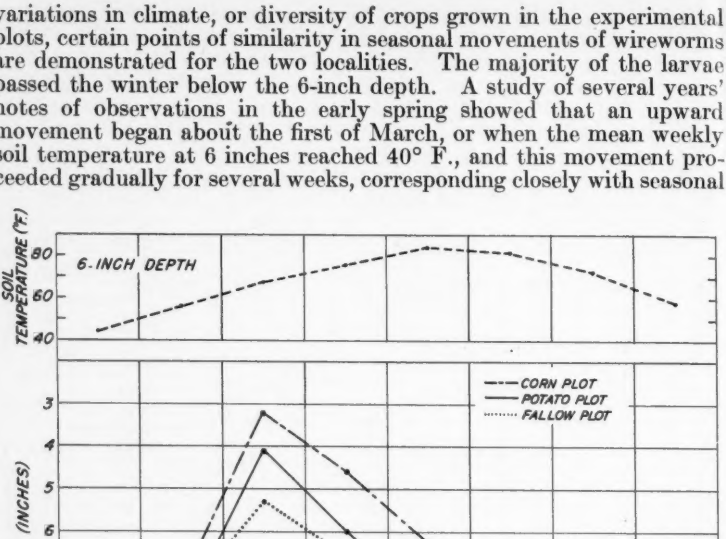

FIGURE 4.—Monthly mean depth of wireworms in the first 18 inches of soil, with monthly mean soil temperatures for the surface 6 inches (6-year average) at Parma, Idaho.

rise in soil temperature. The rate increased sharply after temperatures reached 50°, which was usually about the middle of April. The greatest concentration of the larvae above 6 inches was in May, when conditions in the surface layers of soil approached the seasonal optimum for wireworms. Larval feeding was also heaviest in May and early June, when the larvae were found at various times to be congregated near the soil surface. At the onset of unfavorable conditions of heat and dryness they would go deeper, and move upward again after rains or during periods of cloudy, cool weather.

After the middle of June a definite downward trend was noted. It was most noticeable in fallow plots, where there was no shade or insulation against soil-surface temperatures, which were then approaching their seasonal maxima. At Walla Walla, where observations were made on both silt loam and sandy loam, the larvae retreated more rapidly and to greater depths in soils of the latter type. This was particularly true in June, July, and August, and was presumably due to the greater heat-conducting capacity of the sandy-loam soils, and also to their tendency to dry out rapidly. Soil type appeared to have little bearing on vertical distribution of wire-

worms during April and May, when the surface environment in all types of soil tended to be favorable for the insects.

Temperature again seemed to become a dominant factor in the fall, causing a downward movement of the larvae. At this season, however, surface temperature below the optimum rather than that above it occasioned the movement to lower depths. The wireworms were attracted to the warmer subsoil layers when the mean temperature at 6 inches fell below 60° F.

The vertical distribution of wireworms is directly related to the damage to crops caused by these insects. During March and April, as the wireworms begin to migrate from the subsoil layers into the surface soil, they find seeds and newly germinating plants to feed on. The early-germinating seeds of sugar beet, potato, onion, carrot, pea, and radish, which are planted in March, have usually passed the susceptible stage before the wireworms begin to arrive at the base of the roots of these plants. The seeds of corn, bean, tomato, and cabbage, and onion sets are planted usually early in May, when the greatest concentration of wireworms is found in the seed zone; consequently many seeds and young susceptible seedlings are destroyed.

Every year wireworms thin out stands of many crops that are planted early in May, but in the last week of May or the first week of June a halt is put to these ravages, when the soil surface becomes so warm that the wireworms move down to cooler layers. Seeds planted at this time have a much better chance of surviving the seedling stage.

The opposite is true of the tuber crops that are maturing early in July. Owing to the up-and-down movements of the wireworms between the surface and the plow line, the chances of tuber injury are much greater. A week or 10 days' delay in the harvesting of early potatoes sometimes makes a difference between obtaining a marketable and an unmarketable crop.

In midsummer, when soil temperatures are at their peak and the soil is drying out in the surface layer, a strong downward migration of wireworms occurs. Many of the older wireworms molt at this time, and some transform to the adult stage in July and August. There is much less feeding during these months. Early-fall rains cool and moisten the soil surface, and a few of the wireworms return in September to feed on the fall-planted crops. But with the seasonal decline in temperature activity is retarded, and the wireworms near the surface soon migrate downward again to depths at which they pass the winter.

CONTROL

The vertical distribution of wireworms has a direct relation to control recommendations. When carbon disulfide[9] is used against wireworms, it must be placed a few inches below the soil surface to force its diffusion downward. When placed 3 or 4 inches deep, it diffuses downward but not horizontally in lethal doses, and the poorest kill is usually found above this level. From the data on vertical distribution the best time to treat soil with carbon disulfide would seem to be July, August, and September, when the largest numbers of wireworms are below 6 inches in the soil.

[9] LANE, M. C., and GIBSON, K. E. CARBON DISULPHIDE AS A CONTROL FOR WIREWORMS. Jour. Econ. Ent. 25: 958–967, illus. 1932.

472326—42——2

When a poor-diffusing chemical, such as crude naphthalene, is used, it is desirable to treat the soil when the wireworms are near the surface and easy to reach. In fallow plots it appears that naphthalene fumigation of soil, when the material is applied by plowing and disking, should be most effective in April, May, and June when the majority of the wireworms are in the top foot of soil.

In the control of wireworms by flooding and drying [10] their depth distribution is less important. Flooding must be done when the temperatures are at their highest, and drying requires nearly a whole season.

The best time to attract wireworms to baits is during April, May, and June, when the largest numbers are near the surface. The summer months are unfavorable for baiting work, because of the labor involved in placing baits deep enough in the soil to be fed upon by the larvae.

SUMMARY

Observations on the seasonal vertical distribution of wireworms were made near Walla Walla, Wash., and Parma, Idaho. By arranging these observations regularly in restricted areas of fairly heavy infestations, it was possible to study trends in numbers of wireworms at different depths in relation to soil temperature, moisture, and cropping conditions. A total of from 10 to 20 1-square-foot soil units were examined for wireworms on each observation date. Each unit area was dug in 3-inch layers to a depth of 18 or 24 inches. Observations were made in the following habitats: At Walla Walla in a spinach field of sandy-loam type, and in fallow, alfalfa, and potato fields of silt-loam type; at Parma in fallow, potato, and corn fields of clay-loam type.

In the spring there is an upward migration of the wireworms from the subsoil to the surface layers, when temperatures range from 40° to 68° F. at the 6-inch depth. In the summer under exposed soil-surface conditions, such as fallow or lightly shaded soil, when surface temperatures of 75° F. and above are reached, wireworms leave the surface 3 inches and return to deeper layers. On densely shaded ground, however, wireworms remain in the top 6 inches of soil more consistently and throughout most of the summer. Wireworms move down as the soil becomes dry, and after it becomes wet from rain or irrigation following a dry period they move up again toward the surface.

Careful planning to avoid seeding early in May will tend to minimize the loss of seed of summer crops from wireworm feeding. The late-planted (June) potato has also a greater chance to escape wireworm damage than the early-planted potato.

The vertical distribution of wireworms has a direct relation to control recommendations. A fumigant such as carbon disulfide would be most effective in July, August, and September, whereas a poorer diffusing chemical, such as naphthalene, would be effective during April, May, and June. The time to attract wireworms to baits is also when the largest numbers are in the surface few inches of soil.

[10] LANE, M. C., and JONES, E. W. FLOODING AS A MEANS OF REDUCING WIREWORM INFESTATIONS. Jour. Econ. Ent. 29: 842-850. 1936.

CYTOSPORA ABIETIS, THE CAUSE OF A CANKER OF TRUE FIRS IN CALIFORNIA AND NEVADA[1]

By ERNEST WRIGHT

Associate pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In 1929 cytospora cankers were first observed extensively on white fir (*Abies concolor* Lindl. and Gord.) and red fir (*Abies magnifica* A. Murr.) in the forests of the northern Sierra Nevada. The following year studies were begun to determine the specific identity of the disease, its distribution, its pathogenicity, and the possible reasons for its increased prominence. This paper gives the results of these studies and presents data on environmental and other factors that apparently favor infection.

For a clear understanding of the disease and its distribution, it is desirable to describe briefly the topography of the region and the range of the respective tree species.

TOPOGRAPHY AND RANGE OF HOSTS

The rugged Sierra Nevada extends north and south along the eastern border of the State of California. It lies nearly at right angles to the prevailing storms coming from the Pacific Ocean, and because of its position and great height it exerts a profound influence on the climatic conditions of the east and west slopes. For this reason areas are sometimes locally referred to as east side or west side, depending on their location in respect to the main divide. Annual precipitation on west-side slopes averages approximately 50 percent more than at comparable elevations on the east side. Here the changes in elevation are more abrupt than on the west side, and the average annual precipitation at 6,000 feet is about 25 inches.

White fir extends throughout the Sierra Nevada range but reaches its best development west of the main summit, where the species grows in mixed stands with other coniferous trees at elevations between 4,000 and 7,000 feet above sea level. Frequent associates are ponderosa pine (*Pinus ponderosa* Laws.), sugar pine (*P. lambertiana* Dougl.), and incense cedar (*Libocedrus decurrens* Torr.). At the higher elevations white fir intermingles with red fir. White fir on the east side rarely grows below 5,000 feet and is occasionally found at elevations as high as 8,000 feet. Under east-side conditions, at altitudes between 5,000 and 7,000 feet, white fir occurs with ponderosa pine, Jeffrey pine (*P. ponderosa* var. *jeffreyi* Vasey), and lodgepole pine (*P. contorta* Loud.). Above 7,000 feet it commonly grows in mixed stands with red fir and Jeffrey pine.

Red fir extends to higher altitudes than white fir, and its range is generally favored by a relatively uniform winter precipitation. It commonly grows in pure stands, but it also occurs in association with white fir, Jeffrey pine, sugar pine, and western white pine (*Pinus*

[1] Received for publication June 27, 1941.

monticola Lamb.). In the northern Sierra Nevada it is perhaps the principal tree in the forest belt at elevations between 6,000 and 8,500 feet.

THE CANKER ON WHITE FIR

Several extensive areas where the canker was present on white fir were originally observed in the vicinity of Lake Tahoe at elevations of 6,000 to 6,500 feet. Here the average annual precipitation is approximately 29 inches. All these areas had been seriously disturbed by man; some were cut over nearly half a century ago. Scattered infections were later found on white fir extending along the east side of the Sierra Nevada from Lake Tahoe north, but it was in the immediate vicinity of this lake that the cankers were most abundant (fig. 1). A number of infected white firs have also been observed near

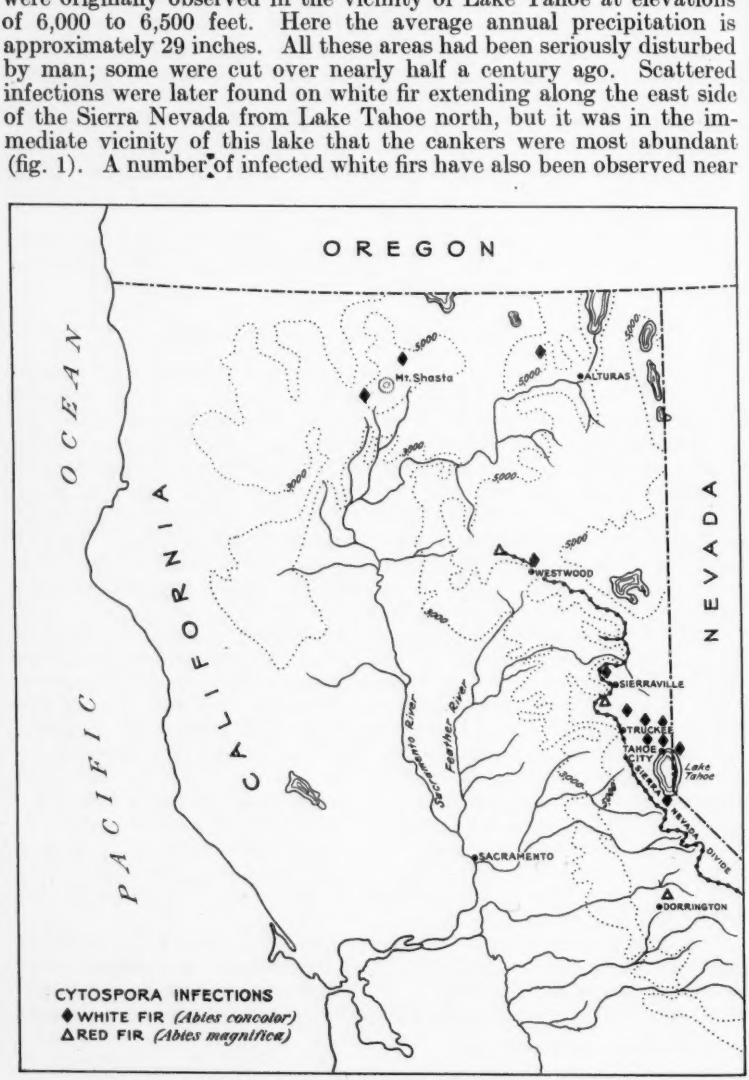

FIGURE 1.—Map showing the observed locations of cytospora infections on white and red firs. Contours and details are omitted from Nevada and from the central and coastal areas of California.

Westwood at elevations of 5,000 to 5,400 feet, a section geographically intermediate between east-side and west-side conditions, which has an average annual precipitation of 23 inches. White firs on the west slope of the Sierra Nevada were free from the canker, but a similar fungus was found there as a saprophyte.

On east-side white fir the first infection usually occurred on a branch of the lower crown, and from this point the fungus spread into the main stem and into the branches immediately above (fig. 2). The appearance of a resin exudate and a characteristic resin infiltration of the bark were the first noticeable symptoms of infection. The cankers, elliptical in outline, extended both lengthwise and around the branch or stem. Yellow spore horns issued from raised pycnidia, generally in the late spring and early summer, and were particularly abundant after periods of precipitation. Later, a copious flow of resin appeared (fig. 3).

FIGURE 2.—A white fir infected with cytospora canker, showing characteristic dying of the lower branches. The upper crown was still healthy and uninfected. Broken line separates infected from healthy branches.

Infections were most frequently found on branches less than 1 inch in diameter. The disease kills by girdling, and it takes from 1 to several years before a girdle is completed, depending upon the circumference of the part infected. During drought years the rate of girdling is apparently accelerated, and the disease becomes almost epidemic in extent. A dieback of the branches results when the girdle is completed, causing the foliage to change from its normal green to yellow and finally to reddish brown. Dying and dead branches, therefore, stand out as characteristic flags. The death of additional branches from year to year reduces the vigor of the diseased white firs, and secondary infestations by bark beetles (*Scolytus* and *Pityophthorus* spp.) contribute to the destruction of the weakened trees. Gilgut (*2*) [2] gives a similar description of a disease of spruce caused by *Cytospora kunzei* Sacc.

THE CANKER ON RED FIR

Branch dying in red fir is very common in the northern Sierra Nevada. The dying has been generally attributed to the *Arceuthobium* mistletoe (*A. campylopodum* Engelm. f. *abietinum* (Engelm.) Gill). A fungus, however, has been found commonly associated with the mistletoe hypertrophies (*3*) and occurring only rarely in the absence of mistletoe. Infected branches vary in size up to several inches in diameter. Spore horns and pycnidia similar to those found on white

[2] Italic numbers in parentheses refer to Literature Cited, p. 152.

fir were observed both in the field and on collected specimens, indicating that *Cytospora* might be the pathogen.

IDENTITY AND ISOLATION OF THE CYTOSPORA

On the basis of numerous microscopic examinations and measurements of pycnidial material from white and red firs, the fungus was tentatively identified as *Cytospora abietis* Sacc. The perfect stage was not found in a mature state, and the identity, therefore, cannot be regarded as final, although the pycnidia and spores of the imperfect stage check closely with the descriptions given in the literature. Sections of pycnidia were compared microscopically with sections of authentic *C. abietis* fruit bodies on other hosts and were found to be similar.[3] No comparisons were made with European material. Seymour (*8*) lists only *Cytospora pinastri* Fr. on *Abies* sp. The *Cytospora* in question is definitely not *C. pinastri*, since the spores are smaller, the spore horns are yellow rather than white, and the fungus is caulicolous instead of foliicolous. There is no record of any *Cytospora* on either white or red fir in the Mycological Collections of the Bureau of Plant Industry, but Hedgcock[4] has reported *C. abietis* on white fir in Oregon.

FIGURE 3.—A typical cytospora stem canker of white fir.

Recently infected branches of white and red fir bearing pycnidia were cut into small pieces, thoroughly washed with sterile water, and placed in moist chambers at room temperatures. After a short period of incubation spore horns exuded from the viable material, and these were used for spore dilutions from which streak cultures were made in agar plates. Pure cultures were obtained by isolating germinating spores under the microscope and transferring them to malt-agar slants (pH 5.5). The colonies which developed from these transfers were later used to make inoculations to test the pathogenicity of the fungus.

[3] Dr. J. C. Gilman, professor of botany at Iowa State College, examined a number of specimens sent to him by the writer and states that so far as he can determine from the material at hand the fungus is probably *Cytospora abietis* Sacc. Dr. Gilman also states that the *Cytospora* on *Abies concolor* and *A. magnifica* is undoubtedly the same species.

[4] HEDGCOCK, GEORGE G. NOTES ON THE DISTRIBUTION OF SOME FUNGI ASSOCIATED WITH DISEASES OF CONIFERS. U. S. Bur. Plant Indus., Plant Dis. Rptr. 16: 28–42. 1932. [Mimeographed.]

PATHOGENICITY TESTS

SLIT INOCULATIONS

Slit inoculations were at first employed to test the pathogenicity of *Cytospora abietis* on white firs. Ten potted white firs placed in the open at Berkeley, Calif., were used in these tests. Five of these were transplanted directly from the woods, and the others were procured from a California nursery. Nine slit inoculations were made in the bark of the plants from the woods. The inoculum, a small piece of malt agar containing *C. abietis* mycelium from a pure culture, was inserted in a slit cut in the bark with a sterile scalpel. The part inoculated was wrapped with cotton and waxed paper to retard drying. Only 2 of the inoculations resulted in the development of a canker, and both of these occurred on the stem of the white fir that had grown most vigorously prior to transplanting. Of 15 similar slit inoculations made in the 5 commercially grown white firs, none was successful. Twelve check insertions of sterile agar in 5 additional plants resulted only in callus formation.

Since the one individual plant infected had grown the most vigorously prior to transplanting, it appears possible that transplanting weakened this vigorously growing white fir more than the slower growing trees, thus increasing its susceptibility to infection.

Better success was expected from the inoculation of white firs growing in the vicinity of Lake Tahoe, where the fungus occurred naturally. Accordingly, 20 slit inoculations were made in the branches of 5 trees predetermined as free from *Cytospora abietis*. The inoculations were carefully wrapped with moist cotton and waxed paper as before to prevent drying out. All of these inoculations were negative, but 2 out of 10 slit inoculations made in white firs already naturally infected were successful.

CORK-BORER INOCULATIONS

A different method of inoculation was then tried, hereafter referred to as the cork-borer method (*12*). The branch or stem to be inoculated was first thoroughly washed with 70-percent alcohol, after which a No. 2 cork borer was forced through the bark down to the xylem and withdrawn, retaining the bark disk in the barrel of the borer. The inoculum, consisting of a small piece of malt agar containing mycelium from a pure culture of *Cytospora abietis*, was deposited in the borer hole with a sterile needle and the disk immediately replaced by forcing it from the barrel directly into position with a plunger. A strip of waterproof adhesive tape was then placed over each disk and wrapped around the branch or stem to hold the core in position and to prevent drying as far as possible.

Several of the white firs that previously had been unsuccessfully inoculated by the slit method were reinoculated by the cork-borer method to compare the effectiveness of the two methods. Trees not previously inoculated were also included in these tests. No attempt was made to follow Koch's postulates. It was considered more important to make a large enough number of inoculations to offset the possibility of natural infection. An inoculation was not regarded as successful unless a definite canker with pycnidia developed around the inoculation court. These inoculations were all made in the late spring and early summer, since this appeared to be the most favorable

time for sporulation and natural infection. The results of the tests are summarized in table 1.

On the basis of these results, the cork-borer method of inoculation proved a satisfactory way of infecting white firs with *Cytospora abietis* growing under natural conditions. The success of the method is probably due to the gradual dying of the disk of bark, which furnishes a suitable substratum for starting infection. The fungus, once established on the bark disk, spreads from this nucleus to the surrounding healthy cambium. The fact that the slit inoculations were generally unsuccessful, while the cork-borer inoculations gave good results, indicates that *C. abietis* is a semiparasite, requiring weakened tissue in which to become established. Certain individual trees, however, showed a natural resistance to infection; for example, on 5 white firs inoculations by the cork-borer method were mostly negative. These firs were adjacent to heavily cankered trees, yet out of the total of 22 cork-borer inoculations made on them, only 3 were positive. On 3 similar trees nearby, which had been infected naturally, 39 out of a total of 44 inoculations were successful.

TABLE 1.—*Results of Cytospora abietis inoculations on white fir*

Condition of host, part inoculated, and method of inoculation	Trees	Inoculations	Infections [1]	
	Number	*Number*	*Number*	*Percent*
Healthy trees	5			
Branches, by slit method		20	1 (?)	5.0
Branches, by cork-borer method		22	3	13.6
Naturally infected trees	17			
Branches, by slit method		10	2	20.0
Branches, by cork-borer method		136	129	94.9
Stem, by cork-borer method		9	8	88.9
Total	22	197	143	72.6

[1] Successful inoculations were determined on the basis of definite cankers and the presence of pycnidia.

Branch cankers originating from internode inoculations extended most rapidly away from the nearest node. The longitudinal growth was about twice as fast as the growth around the circumference of the branch. Stem cankers in the internodes developed somewhat faster downward from the point of inoculation than upward. Infections arising from inoculations made directly in the nodes on either branches or main stems grew less rapidly longitudinally than those in the internodes, so that the cankers appeared more nearly round in outline than those originating from internode inoculations. On plants growing under forest conditions, it took from 12 to 15 months for *Cytospora abietis* to completely girdle branches one-half inch in diameter. In favorable years, however, the girdling action may be more rapid than this, as for example in 1929, when the disease first attracted attention.

CROSS INOCULATIONS

Isolates of *Cytospora abietis* from white fir and from red fir, when placed in the same Petri dish, showed a tendency to mutual aversion. The isolates from red fir grew more rapidly and less densely than those from white fir. These differences, together with the specific occurrence of each fungus, suggested that possibly one was a more virulent strain than the other. To test this possibility a number of cross inoculations

were made by the cork-borer method with pure cultures of *C. abietis* obtained from each host. The trees selected for the tests were at an elevation of 7,000 feet, where white fir and red fir grew intermixed. Precautions were taken to eliminate cross contamination by using separate inoculation needles and by making all the inoculations with the *Cytospora* isolate from white fir before proceeding with that from red fir. Some of the inoculations were made in mistletoe hypertrophies, while others were made in healthy branches and stems. Before inoculation the bark was thoroughly washed with 70-percent alcohol, and after inoculation adhesive tape was employed as before to hold the bark cores in place. The results of these inoculations are given in table 2.

TABLE 2.—*Results of cross inoculations with Cytospora abietis*

Host and part inoculated	Inoculations with white fir isolate		Inoculations with red fir isolate		Total inoculations	Total infections	
	Inoculations	Infections	Inoculations	Infections			
White fir:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Branches	16	15	24	24	40	39	97.5
Stems	4	2	4	3	8	5	62.5
Mistletoe hypertrophies [1]	24	15	0	0	24	15	62.5
Total	44	32	28	27	72	59	81.9
Red fir:							
Branches	9	7	9	6	18	13	72.2
Stems	3	1	2	1	5	2	40.0
Mistletoe hypertrophies [1]	23	13	10	10	33	23	69.7
Total	35	21	21	17	56	38	67.9

[1] Caused by *Arceuthobium campylopodum* Engelm. f. *abietinum* (Engelm.) Gill.

From the results of these cross-inoculation tests it appears that both isolates readily infect either host. Measurements of the cankers formed gave no indication that either of the isolates grew faster than the other. There also appeared to be relatively little difference in virulence between the two strains when judged on the basis of the number of branches killed. The combination of *Cytospora abietis* with mistletoe sometimes hastens the death of hypertrophied branches of both species. White fir generally seemed to be somewhat more susceptible to infection by *C. abietis* than red fir, but the number of inoculations was not sufficient to prove this point. Cross inoculations failed to explain satisfactorily why branch dying in red firs has been so pronounced in certain sections of the northern Sierra Nevada.

Other factors that possibly predispose white and red firs to natural infection by *Cytospora abietis* are discussed in following sections of this paper.

FACTORS PREDISPOSING TO INFECTION BY CYTOSPORA ABIETIS

The rapid spread of weakly parasitic fungi like *Cytospora* has been ascribed by investigators to host decline. Predisposing factors such as drought and fire have been frequently cited as contributory agents (*1*, *4*, *5*, *6*, *7*). In studying *Cytospora abietis* an attempt has been made to analyze some of the more evident predisposing factors that may have aided in the flare-up of the cytospora disease in the Sierra

Nevada. The most important of these has been the effect of drought on white fir.

During the period 1924–30, inclusive, there were two distinct droughts in the Sierra Nevada. The year 1924 was characterized by one of the most severe and widespread droughts since the beginning of reliable records. This resulted in extremely small radial growth for many forest species (*11*). For that year the seasonal precipitation at Tahoe, Calif., was only 14.18 inches, as compared with a normal mean of 29.41 inches. Then followed several seasons when the precipitation was about normal. However, in 1929 it dropped to 20.84 inches, resulting in a second severe drought within 5 years. It was during the drought season of 1929 that the cytospora disease of white fir was first observed to be killing large numbers of branches and occasional trees in what appeared to be thrifty stands of reproduction.[5] For the entire period 1924–30, the late fall and winter precipitation was especially deficient as compared with that of the years succeeding this period. Since 1930 the rate of spread of *Cytospora abietis* has gradually decreased, even though during the 4 years 1931–34 there was less than normal precipitation during the growing season. This was compensated for, however, by nearly average precipitation during the months of December and January, the normal period of moisture accumulation.

To study the effect of droughts on the growth of white firs, increment borings were taken in 1934 from 50 sound and 50 cytospora-infected trees located along a transect line on east-side sites at elevations of 6,300 to 6,500 feet. The borings were obtained from the main trunk at 1 foot from the ground, and the widths of the annual rings were determined by measurements under a hand lens. Trees with large stem cankers, indicating old infections, were avoided, since such infections may possibly have had an influence on rate of growth.

It was found that prior to the 1924 drought diameter growth had been more rapid for the infected white firs than for the sound ones. From that year on, the width of the annual rings decreased more rapidly in the diseased than in the healthy trees. It is possible that along with the reduction in increment due to drought there was also a decline in vigor in the formerly fast-growing white firs, thus rendering such trees more susceptible to infection by *Cytospora*. The annual increment for all trees had been small, averaging only 1.9 mm. for the uninfected firs.

Supplementing the above study, 51 additional increment cores were compared at the end of the 1937 growing season.[6] The average annual rate of growth at 1 foot from the ground was again determined for the period 1924–30, as well as for the years preceding and succeeding this drought period up to 1937.

The data presented in table 3 tend to verify previous findings that the most heavily infected white firs grew the fastest prior to the droughts of 1924 and 1929 but suffered the greatest reduction in increment for the 1924–30 period. It is doubtful, however, whether these data are statistically significant, since the growth differences were so small.

[5] WAGENER, W. W. TREE DYING FROM PATHOLOGICAL CAUSES IN THE VICINITY OF LAKE TAHOE. Division of Forest Pathology. 1930. [Unpublished report.]

[6] These increment cores were obtained for the writer by Dr. W. W. Wagener, of the Division of Forest Pathology, stationed at San Francisco.

TABLE 3.—*Comparison of growth rates of Cytospora-infected and noninfected white firs (Tahoe region)*

Degree of infection	Trees	Average annual growth		
		1909–23	1924–30	1931–37
	Number	*Mm.*	*Mm.*	*Mm.*
None	18	1.3	1.2	1.3
Light (old)	8	1.3	1.2	1.2
Moderate (old)	7	1.5	1.3	1.1
Heavy (old)	10	1.7	1.3	.9
Light (recent)	8	1.5	1.6	1.4

On the basis of analyses of the increment cores, it appears that the white firs which had grown slowly prior to the 1924–30 period were less adversely affected by the droughts of 1924 and 1929 than trees which had grown more rapidly before these droughts. The formerly more rapidly growing trees, therefore, proved somewhat more susceptible to *Cytospora abietis*, presumably because of reduced vigor. Drought may account, at least partially, for the increase of the cytospora disease in white fir growing on east-side sites, where moisture deficiencies have been most noticeable.

In certain localities in the Sierra Nevada, ground fires have brought about widespread infection of white firs by *Cytospora abietis*, similar to that reported for other species of trees (*1*, *6*). Of special interest is the work of Togashi (*10*), who found that apple trees were most readily inoculated with *Valsa mali* Miyabe and Yamada, when the inoculation wounds were made with a hot scalpel or through bark that had been burned.

The scorching of young white firs by ground fires not only weakened the trees but also resulted in patch-killing of the relatively thin bark, causing it to shrink and crack. These wounds on fire-weakened trees apparently became infection courts for *Cytospora abietis*. The fire-scorched firs, therefore, have provided a harbor for the fungus and have perpetuated it until predisposing factors have rendered adjacent unburned stands susceptible to infection. This may be one explanation for the sudden flare-up of this disease following the droughts of 1924 and 1929, which were also bad fire years.

An increase in beetle population, common during drought years, resulted in an unusual number of exploratory holes into which the beetles may have carried the infection.[7] The beetle galleries also weaken infested branches and may, therefore, help to predispose them to infection. In nature, mistletoe hypertrophies, especially on red fir branches, are commonly infested with the bark-engraver beetle, *Scolytus subscaber* Lec. (*9*). In a previous publication (*13*) it has been shown that this beetle carries a staining fungus which kills the cambium adjacent to the beetle galleries. Thus the staining fungus, together with the galleries, may weaken infested branches to the point where they also become readily susceptible to infection by *Cytospora abietis*. The effect of this combination, therefore, may explain the unusually heavy mortality of red-fir branches as previously described.

Aphid colonies also appear to reduce the vigor of individual branches. Such colonies have been repeatedly observed, particularly on the under

[7] In one case the writer succeeded in isolating *Cytospora abietis* from a fresh beetle hole before there was any sign of canker development.

side of the lower limbs of white fir. In several cases the remains of old aphid colonies were found on branches recently infected by *Cytospora abietis*. Aphid colonies were observed in midspring, about the time when *C. abietis* spore production is greatest. The ants associated with the aphid colonies have also been seen to pass over sporulating cytospora pycnidia on their way to and from aphid colonies, and may in this way transfer the spores to areas where infection can readily take place. While this evidence is admittedly circumstantial, it is based on observations made frequently enough to preclude the factor of chance. This is one explanation for the appearance of isolated branch cankers that cannot be directly traced to other causes.

It is concluded from these studies that *Cytospora abietis* is normally present in the forests of the Sierra Nevada as a semiparasite and that it becomes of importance only when the firs are weakened by drought, fire, insects, or other factors.

SUMMARY

A canker of white fir (*Abies concolor*) and red fir (*A. magnifica*) caused by *Cytospora abietis* has been observed in the forests of the northern Sierra Nevada. Typical symptoms are an early resin infiltration of the bark, followed by the development of a canker and the production of yellow spore horns. Infections on white fir begin in the lower branches and may gradually progress into the stem and branches above. On red fir the infections are commonly found in association with mistletoe hypertrophies.

Slit inoculations were generally unsuccessful in transmitting the disease to white fir. Much better results were obtained by a cork-borer method of inoculation. Cross inoculations from red fir to white fir and vice versa have shown that the two cytospora isolates attack both hosts. There appeared to be no significant difference in virulence of either so-called strain of the fungus. The fungus is classed as a semiparasite, usually infecting through artificial wounds.

The comparison of diameter growth from increment borings taken from healthy and cytospora-diseased trees suggested that drought may be an important factor, particularly in weakening fast-growing white firs previous to infection. Fire-scorched trees appear to aid in perpetuating *Cytospora abietis* during periods between droughts. Other agents, such as bark beetles, aphids, and ants, probably spread the disease locally and predispose individual branches to infection. The fungus appears to be endemic in the forests of the Sierra Nevada and only assumes epidemic proportions when the true firs are weakened by environmental or other factors.

LITERATURE CITED

(1) Dearness, John, and Hansbrough, J. R.
1934. cytospora infection following fire injury in western British Columbia. Canad. Jour. Res. 10: 125–128.

(2) Gilgut, C. J.
1936. cytospora canker of spruces. Natl. Shade Tree Conf. Proc. 12: 113–119.

(3) Gill, L. S.
1935. arceuthobium in the united states. Conn. Acad. Arts and Sci. Trans. 32: [111]–245, illus.

(4) HUBERT, ERNEST E.
1920. OBSERVATIONS ON CYTOSPORA CHRYSOSPERMA IN THE NORTHWEST. Phytopathology 10: 442–447.
(5) LONG, W. H.
1918. AN UNDESCRIBED CANKER OF POPLARS AND WILLOWS CAUSED BY CYTOSPORA CHRYSOSPERMA. Jour. Agr. Res. 13: 331–345, illus.
(6) POVAH, ALFRED H. W.
1921. AN ATTACK OF POPLAR CANKER FOLLOWING FIRE INJURY. Phytopathology 11: [157]–165, illus.
(7) SCHREINER, ERNST J.
1931. THE ROLE OF DISEASE IN THE GROWING OF POPLAR. Jour. Forestry 29: 79–82.
(8) SEYMOUR, ARTHUR BLISS.
1929. HOST INDEX OF THE FUNGI OF NORTH AMERICA. 732 pp. Cambridge, Mass.
(9) STRUBLE, G. R.
1937. THE FIR ENGRAVER BEETLE, A SERIOUS ENEMY OF WHITE FIR AND RED FIR. U. S. Dept. Agr. Cir. 419, 16 pp., illus.
(10) TOGASHI, KOGO.
1924. SOME STUDIES ON A JAPANESE APPLE CANKER AND ITS CAUSAL FUNGUS, VALSA MALI. Hokkaido Imp. Univ., Jour. 12: [265]–324, illus.
(11) WAGENER, WILLIS W.
1940. THE PHYSICAL EFFECTS OF DROUGHT ON SHADE TREES. Western Shade Tree Conf. Proc. 7: 21–30, illus.
(12) WRIGHT, ERNEST.
1933. A CORK-BORER METHOD FOR INOCULATING TREES. Phytopathology 23: 487–488.
(13) ———
1938. FURTHER INVESTIGATIONS OF BROWN-STAINING FUNGI ASSOCIATED WITH ENGRAVER BEETLES (SCOLYTUS) IN WHITE FIR. Jour. Agr. Res. 57: 759–773, illus.

SOME MENDELIAN CHARACTERS IN BETA VULGARIS AND LINKAGES OBSERVED IN THE Y–R–B GROUP[1]

By F. V. OWEN, *geneticist*, and GEORGE K. RYSER, *collaborator, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Published data regarding Mendelian inheritance in *Beta vulgaris* L. have been largely limited to characters dependent upon four genetic factors: *B* (for bolting or annual habit), *pl* (for plantainlike leaf venation), *R* (for red plant color), and *Y* (for production and extension of pigment). Keller (*6*) [2] showed a close linkage between *R* and *Y* and summarized previous literature. He also presented evidence for an allelic series, *Y*, Y^r, and *y*, at the *Y* locus and another series, R^t, *R*, and *r*, at the *R* locus. Abegg (*1*) described the leaf-venation character dependent upon the factor *pl* and demonstrated a linkage between *B* and *R*, the annual habit produced by the factor *B* having been previously reported by Munerati (*9*). Munerati and Costa (*10*) described a black or corky root condition which may be the same as that produced by the factor *bl* considered here. A list of characters and gene symbols from unpublished as well as from published reports, including all characters and genes discussed in the present paper, has recently been presented by Abegg (*2*).

The present paper deals with linkage relationships of 10 Mendelian factors and includes brief descriptions of the characters represented.

MATERIAL

The factors *R* and *Y* were secured from diverse sources. Both *R* plants and *r* plants are common to many commercial varieties of sugar beets. The factor *Y* was secured from the Detroit Dark Red variety of garden beets and the Golden Tankard variety of mangels. The source of the factor Y^r, a variation at the *Y* locus, was derived from material described by Keller (*6*) and from the Long Red Mammoth and the Golden Tankard varieties of mangels.

Material carrying the factor *B*, for bolting or annual habit, was obtained from Dr. O. Munerati.[3]

The factors *bl* (for black root), *Cl* (for colored leaf), *Cv* (for colored leaf veins), *pl* (for plantainlike leaf venation), *ru* (for russet root), *Tr* (for trout or spotted leaf), and v_1 (for variegated foliage) interfere more or less with the vigor of the plant and are not common to commercial varieties of sugar beets. Material carrying these seven factors was secured from various sources.[4]

---

[1] Received for publication March 17, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 171.

[3] Director, R. Stazione Sperimentale di Bieticultura, Rovigo, Italy.

[4] *bl*, *Cl*, and *Tr* secured by Dr. F. R. Immer from Dr. F. Schneider, of the Zuckerfabrik Kleinwanzleben at Kleinwanzleben, Germany.

Cv from G. W. Deming, assistant agronomist, Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

pl from Dr. D. A. Pack, formerly associate agronomist, Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

ru and r_1 found as mutant types in curly top resistant varieties of sugar beets (*3*), Salt Lake City strains 621 and 2113, respectively.

ALPHABETICAL LIST OF GENES AND DESCRIPTION OF CHARACTERS AFFECTED

B.—Bolting, or annual habit (*1*). Under greenhouse conditions *B* plants produced seedstalks under long photoperiods (18-hour or 24-hour days) with relatively warm temperature, and *b* plants remained vegetative. Expression clear-cut for data reported, but unpublished data show that after successive backcrosses to nonbolting types, additional or modifying factors cause difficulty in classifying *B* and *b* plants. A possible variation at the *B* locus, which produces a bolting tendency but not a strictly annual habit, described by Owen, Carsner, and Stout (*12*). In the same linkage group as *R* and *Y*.

bl.—Black root, caused by development of corky tissue on the skin of the root (fig. 1, *A*). Viability fair but *bl* plants produced much less corky tissue than others, and some were not easily distinguished from russet (*ru*) plants with much less cork development. No positive indications of linkage with *R*, *Y*, *pl*, or *ru*. (See tables 5 and 6.) Similar or identical character reported by Munerati and

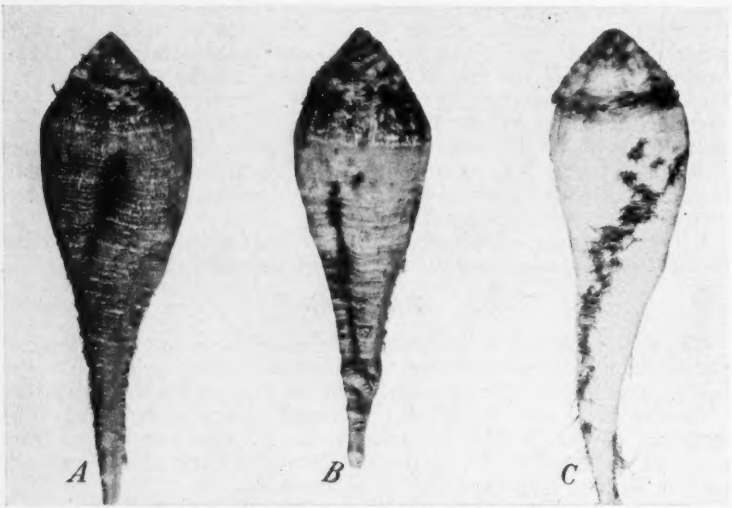

FIGURE 1.—Root characters in *Beta vulgaris: A*, Black root (*bl*); *B*, russet root (*ru*); *C*, normal white root.

Costa (*10*), who consider the character variable and unstable and have reported instances of mosaic spots of corky tissue.

Cl.—Colored leaf, red pigment with *R* and yellow pigment with *r* (fig. 2). Development of pigment largely dependent upon direct sunlight, but a tendency noted for pigment to appear in blotches and principally on the under side of the leaf. *Cl* plants definitely smaller than normal + (or *cl*) plants (see table 7), and handicap in growth appears to be at least roughly correlated with intensity of pigment development. Restricted growth of one-half of the leaf is commonly seen (fig. 2, *C*), with more intense pigment development on one side. Instability at the *Cl* locus suspected because certain plants, selected from types with less development of pigment, bred true for this tendency. In the *Y–R–B* linkage group and very closely linked to *Tr*. (See table 1.)

Cv.—Colored leaf, especially near the veins, red with *R* and yellow with *r* (fig. 3). Expression variable and dominance incomplete. Pigment associated with a glossy condition on upper side of the leaf; leaves usually roll inward (fig. 3, *B* and *C*). This glossy condition and leaf rolling associated with decreased vigor, especially in homozygous *Cv Cv* plants. Pigment pronounced in first two leaves in seedling stage (fig. 3, *D*), also in cotyledons under field conditions. Pigment usually most pronounced near the veins of the leaf but absent from the

midrib of the petiole, where the most pigment develops in *Y* plants. In the field, especially with homozygous *Cv Cv* plants, intensity of pigment development

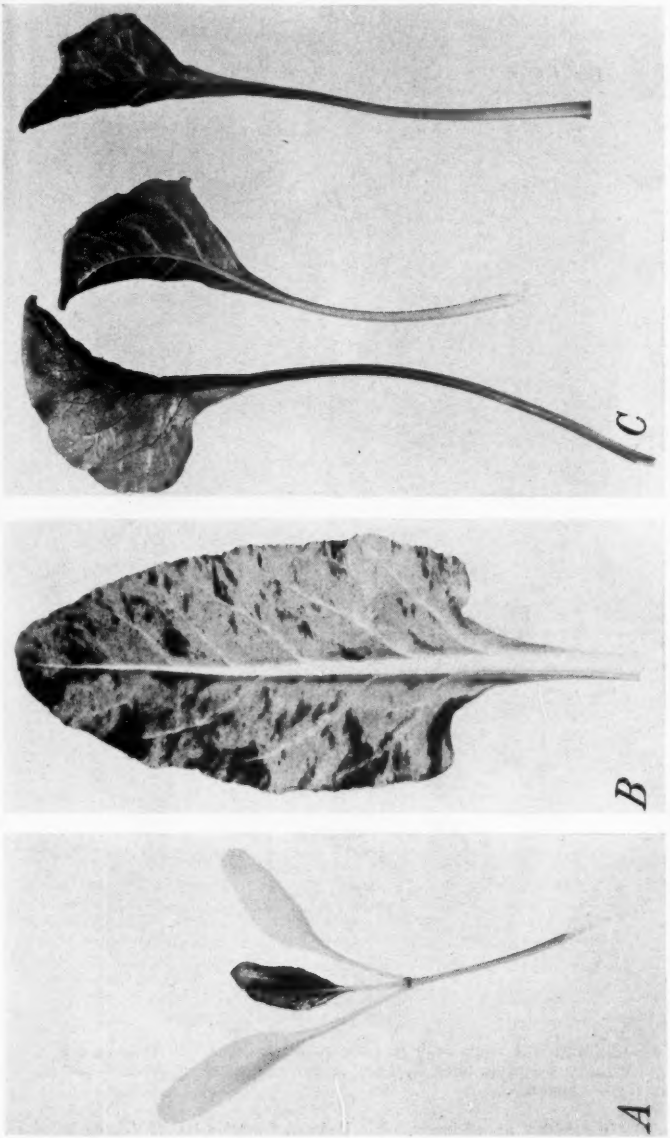

FIGURE 2.—Colored leaf (*Cl*) in *Beta vulgaris*, showing pigment (red) in presence of *R*: *A*, Greenhouse seedling showing red color in first two leaves and lack of pigment in the cotyledons; *B*, under side of leaf showing typical blotches of pigment; *C*, three leaves showing tendency for distorted growth.

is increased and may spread over the surface of the leaf, producing a solid color except in the midrib, which still remains free from pigment. In the *Y–R–B* linkage group and closely linked to *Tr*. (See table 1.)

472326—42——3

pl.—Plantainlike leaf venation (*1*). Most *pl* plants are readily classified, but occasional intermediate types are difficult to classify. Experience indicates

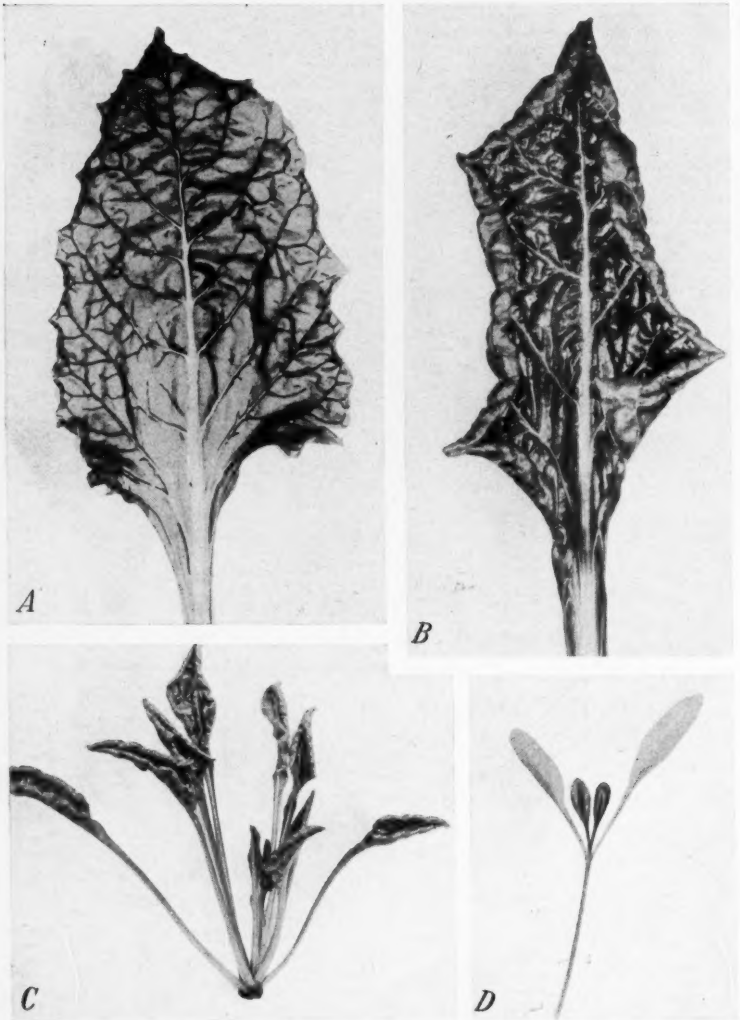

FIGURE 3.—Colored leaf veins (*Cv*) in *Beta vulgaris*: *A*, With *R*; *B* and *C*, with *r*; *D*, greenhouse seedling, with *R*, showing more pigment in the first two leaves than in the cotyledons.

considerable instability at *pl* locus. No linkage found with *R* (*1*) or with *bl*. (See table 5.)

R.—Red plant color, especially in hypocotyls of seedlings and crown buds of older plants, yellow with *r*. No difference between *R* and *r* plants (*11*) in viability

and vigor, but see *Cv* for exception. Both *R* and *r* plants are common to most sugar-beet varieties. The red pigment, betanin (*13*), sometimes fades out completely when a plant initiates a seedstalk. In the same linkage group as *Y* and *B*.

R^t.—More intense development of pigment, especially in the form of red stripes at the base of the petioles. Character found and described by Keller (*6*); also found by the writers in Large Mammoth variety of mangels.

ru.—Russet root, caused by development of corky tissue in the skin of the root (fig. 1, *B*). Similar to *bl*, but *ru* plants developed less cork and were more vigorous than *bl* plants. (See table 7.) Viability good and classification easy under field conditions by end of season. Early in season or under greenhouse conditions classification sometimes difficult. No indications of linkage with *R* or *Y* (see table 5) or with *bl* (see table 6).

Tr.—Trout or spotted leaf, red pigment with *R* and yellow with *r* (fig. 4). Classification of *Tr R* plants with regard to *Tr* easier than with *Tr r* plants. Young plants often more easily classified than old plants. In the post-seedling stage, *Tr* plants are readily classified by a heavy production of pigment on the first two true leaves. The pigmentation at this stage is more intense than at any other stage. Spots in old leaves may be difficult to see. Sometimes difficult to classify in presence of *Cl*, *Cv*, or *Y*. A characteristic of *Tr* plants is development of pigment in leaves in darkness. As the chlorophyll fades from leaves in darkness the red or yellow spots on *Tr* plants are much more striking. This is in decided contrast to behavior of leaves of *Cl* plants, which lose their pigment in darkness. In the *Y–R–B* linkage group, and very closely linked to *Cl* and *Cv*. (See table 1.)

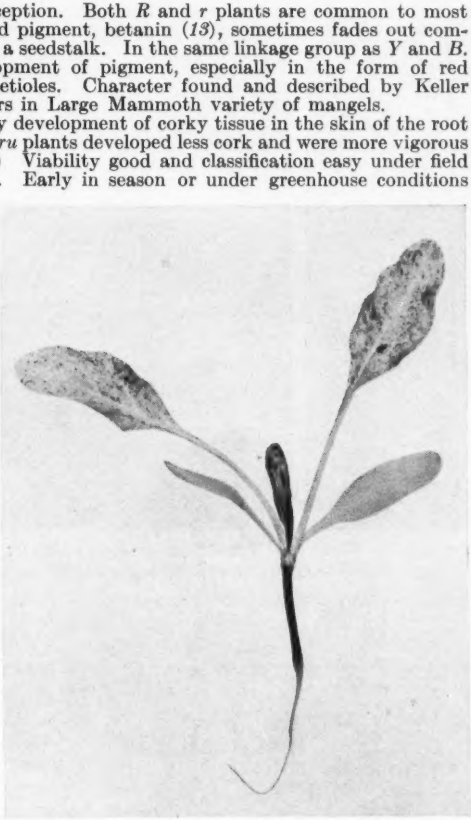

FIGURE 4.—Trout or spotted leaf (*Tr*) in *Beta vulgaris*, showing red spots in presence of *R* and lack of pigment in cotyledons.

v_l.—Variegated foliage, white with irregular blotches of green (fig. 5). Viability fair if seedlings are not crowded. Vigor not reduced in early cotyledon stage because cotyledons develop normally without chlorophyll deficiency. Leaves lack chlorophyll and plant vigor is decidedly reduced, but many v_l plants have flowered and produced seed when given adequate care and space. Experience shows considerable instability at the v_l locus. In the *Y–R–B* linkage group at the opposite end from *Y*.

Y.—Heavy production and extension of pigment, red with *R* and yellow to orange with *r*. Most of the pigment is in the root but is also extended to foliage, especially in midrib of petiole and larger leaf veins (fig. 6). Viability normal and vigor not reduced. (See table 7.) Much variation in expression noted, indicating not only variability due to environment but also variability due to accessory factors. Fullest development of pigment secured under field conditions with bright sunlight, cool temperatures, and plants widely spaced, but considerable pigment develops in darkness, as noted with germinating seedlings or leaf tissue from beets in storage. Under conditions for fullest development of pigment, *Y* may be more or less epistatic to color factors *Cl*, *Tr*, and *Cv*. *Y* factor found in red garden beets and many varieties of mangel-wurzels. In the same linkage group as *R* and *B*.

FIGURE 5.—Variegated foliage (v_1) in *Beta vulgaris: A*, Seedling showing normal green cotyledons and variegated true leaves; *B*, typical plant.

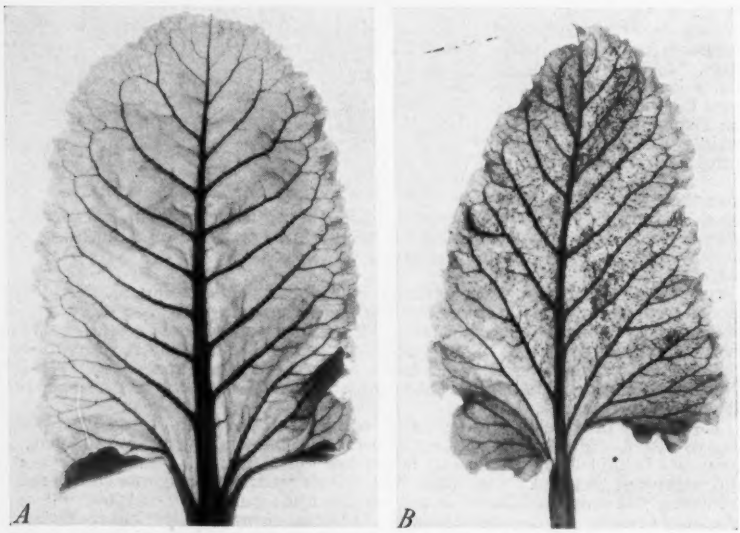

FIGURE 6.—Extension of pigment (*Y*) in *Beta vulgaris: A*, Larger veins highly colored; *B*, extension of pigment to tissue between the veins.

Y^r.—Similar to *Y* but less development of pigment, and production of pigment largely restricted to the root (*6*). Skin color of root usually lemon yellow. Easily classified under field conditions, but under greenhouse conditions sun treatment (*6*) may be necessary to bring out the color.

METHODS

Appropriate crosses were made by exchanging paper bags over the inflorescence. By using self-sterile plants for the female parent it was possible to make crosses without emasculation of flowers. In making backcrosses it was important to check carefully to see that the female plants were strongly self-sterile in order to avoid a mixture of F_2 and backcross individuals from a partially self-fertile female plant. In order to judge whether any significant amount of selfing took place, backcross data from recessive and dominant female parents are reported separately.

Data reported for F_2 plants were obtained from progenies arising from crossing two heterozygous self-sterile plants or by selfing self-fertile plants. Knowledge of a single factor for self-fertility (unpublished data) made it possible to make plans in advance to have self-fertility when selfing was desired and to avoid self-fertility when it was not desired.

Populations were grown either in the greenhouse or the field, according to the amount of seed available and the requirements for classification.

Classification of plants for the presence or absence of the factor *B*, for bolting or annual habit, was accomplished by growing them in a greenhouse with an 18-hour or 24-hour photoperiod and under relatively warm temperature. In this environment experience showed that the *b* plants, and plants from a biennial variety grown in each case for a check, remained vegetative.

With populations segregating for the factor v_1, for variegated foliage, care was taken to separate the v_1 plants from the normal + (or V_1) plants in the seedling stage to permit better growth of the v_1 plants. Otherwise the v_1 plants were unable to compete and survive against the competition from the more vigorous normal plants.

Satisfactory classifications for populations segregating for factors *Cl* and *Tr* in the presence of *Y* were made by working with young plants to avoid the masking effect of the intense pigment produced by the *Y* factor. Greenhouse plants were preferred to plants grown in the field when it was desired to avoid intense pigment development.

In order to establish the linear order in the *Y–R–B* linkage group, characters were combined in hybrids to give 3- and 4-point linkages. (See tables 3 and 4.)

THE Y–R–B LINKAGE GROUP

Two-point linkages are presented in table 1, where at the extreme left the respective genes are listed corresponding to *X* and *Y* in the heading. Phenotypes are represented by *a*, *b*, *c*, and *d*, which correspond to *XY*, *Xy*, *xY*, and *xy* respectively. Linkage phases are represented by "CB" for coupling in backcross, "RB" for repulsion in backcross, "CF_2" for coupling in F_2, and "RF_2" for repulsion in F_2. Under "Female parent," "Rec." stands for recessive, and "Dom." for dominant. Recombination percentages were calculated by the

method of maximum likelihood (*7*). In backcrosses the maximum likelihood value was obtained by the usual method of dividing the number of recombinations by the total number of individuals. In F_2 populations the maximum likelihood value was obtained by the solution of the equation

$$P=\frac{(a-2b-2c-d)\pm\sqrt{(a-2b-2c-d)^2-8dn}}{2n}$$

where $P=p^2$ for repulsion and $(1-p)^2$ for coupling, and p is the recombination fraction, assuming equal crossing over in both sexes, and $n=a+b+c+d$, or the total number of individuals.

Use of the maximum likelihood method facilitated combining information from backcross and F_2 populations by the method described by Mather (*7*). A combined maximum likelihood value was obtained for the recombination percentage between R and v_1, which involved consideration of three equations as follows:

$$+\frac{\text{Recombinations}}{p}-\frac{\text{parental combinations}}{1-p}=0, \text{ for backcross populations}$$

$$-\frac{a}{3-2p+p^2}+\frac{b+c}{2p-p_2}-\frac{d}{1-2p+p^2}=0, \text{ for } F_2 \text{ with factors in coupling phase}$$

$$+\frac{ap}{2+p^2}-\frac{(b+c)p}{1-p_2}+\frac{d}{p}=0, \text{ for } F_2 \text{ with factors in repulsion}$$

The combined estimate was made by taking the sum of these three equations and equating to 0. Solution was accomplished by the method of arithmetic approximation described by Mather (*7*), a method which also gives an arithmetic solution for the variance and standard error. The solution is 17.7 ± 0.83 percent recombinations between R and v_1 (table 1).

A combined estimate for backcross and F_2 data was also obtained for the recombination percentage between R and B by utilizing data published by Abegg (*1*) and combining them with data obtained by the writers. This involved consideration of the first two equations listed above, and the solution is 15.9 ± 0.58 percent recombinations between R and B (table 1).

Standard errors follow a plus-or-minus sign ($\pm$) after each recombination percentage listed in table 1. These standard errors were taken, except as already noted for combined maximum likelihood values, from unpublished tables furnished by Immer, which differ from Immer's published tables (*5*) in that standard errors are given rather than probable errors. Although Immer's tables for F_2 data were calculated for use with the product method (*5*), their use with the maximum likelihood method is legitimate because the variances for the recombination fraction by the two methods are identical (*7*). The standard errors were taken without interpolation to correspond to the nearest 0.5 percent recombinations.

In order to study the possibility of having obtained some selfing of female plants in backcrossing, all backcross data (table 1) are presented separately according to whether the female parent was recessive or dominant. If, for instance, a recessive *r r y y* plant is pollinated with pollen from a double heterozygous *R r Y y* plant, the backcross population should segregate with 1 : 1 ratios for presence

TABLE 1.—*Linkage data, Y–R–B group*

Genes (*X Y*)	Linkage phase	Female parent	Families	Individuals					Recombinations	
				XY (*a*)	*Xy* (*b*)	*xY* (*c*)	*xy* (*d*)	Total (*n*)		
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
B Cv	RB	Rec.	1	12	152	145	36	345	48	13.9 ± 1.87
	RB	Dom.	2	75	564	602	81	1,322	156	11.8 ± .89
Total			3					1,667	204	12.2[2] ± .80
B R	CB	Rec.	5	234	42	62	277	595	104	17.5 ± 1.56
	CB	Dom.	2	538	101	106	577	1,322	207	15.7 ± 1.00
	CB		7	286	56	54	294	[1] 690	110	15.9 ± 1.40
	CF_2		7	1,095	120	103	276	[1] 1,594		15.3 ± 1.00
Total			21					4,201		[3] 15.9 ± .58
B Y	RB	Rec.	4	42	131	160	67	[3] 400	109	27.3 ± 2.23
B v_1	RB	Rec.	1	6	21	64	34	[3] 125		
	RB	Dom.	2	18	22	149	93	[3] 282		
Total			3	24		213		[4] 237	24	10.0 ± 1.95
Cl R	CB	Rec.	11	684	20	32	669	1,405	52	3.7 ± .49
	CB	Dom.	4	250	3	8	248	509	11	2.2 ± .62
	RB	Rec.	6	46	1,023	1,071	49	2,189	95	4.3 ± .44
Total			21					4,103	158	3.8 ± .31
Cl Tr	RB	Rec.	6	0	946	989	3	1,938	[5] 3	
	RB	Dom.	2	0	163	155	0	318	0	
Cl Y	CB	Rec.	2	162	16	11	179	368	27	7.3 ± 1.37
	RB	Rec.	7	75	571	546	86	1,278	161	12.6 ± .93
	RB	Dom.	4	18	157	164	22	361	40	11.1 ± 1.65
Total			13					2,007	228	11.4 ± .71
Cv R	RB	Rec.	2	9	226	230	18	483	27	5.6 ± 1.04
	RB	Dom.	4	41	983	977	40	2,041	81	4.0 ± .43
Total			6					2,524	108	4.3 ± .41
Cv P	CF_2		5	1,708	36	31	525	2,300		3.0 ± .36
Cv Tr	RB	Rec.	3	0	410	334	3	747	[5] 3	
Cv v_1	CF_2		5	1,610	134	206	350	[3] 2,300		16.5 ± .86
R Tr	CB	Rec.	9	1,226	62	48	1,220	2,556	110	4.3 ± .41
	CF_2		1	459	19	12	137	627		5.1 ± .90
	RF_2		1	315	205	137	0	[3] 657		
R v_1	CB	Rec.	1	36	10	14	51	111	24	21.6 ± 3.90
	CB	Dom.	1	75	12	24	59	[3] 170	36	21.2 ± 3.12
	RB	Dom.	2	42	100	125	15	[3] 282	57	20.2 ± 2.38
	CF_2		9	2,231	195	284	525	3,235		16.3 ± .73
	RF_2		1	201	93	83	3	380		18.9 ± 4.90
Total			14					4,178		[3] 17.7 ± .83
R Y	CB	Rec.	5	346	26	16	349	737	42	5.7 ± .84
	CB	Dom.	2	63	6	5	45	119	11	9.2 ± 2.62
	RB	Rec.	12	106	1,027	978	107	2,218	213	9.6 ± .62
	RB	Dom.	6	68	697	757	58	1,580	126	8.0 ± .68
R Yr	RB	Rec.	7	7	145	171	17	340	24	7.1 ± 1.38
	RB	Dom.	2	15	107	116	8	246	23	9.3 ± 1.87
Total			34					5,240	439	8.4 ± .39
Tr Y	CB	Rec.	2	70	8	12	71	161	20	12.4 ± 2.61
	CB	Dom.	2	98	13	10	111	232	23	9.9 ± 1.97
	RB	Rec.	4	52	274	404	54	[3] 784	106	13.5 ± 1.22
Total			8					1,177	149	12.7 ± .96
v_1 *Y*	CB	Rec.	1	49	21	21	46	137	42	30.7 ± 3.94
	CB	Dom.	2	127	21	40	94	282	61	21.6 ± 2.45
Total			3					419	103	24.6 ± 2.10

[1] Abegg's (*1*) data.
[2] Derived from combined maximum likelihood values.
[3] Significant departures from theoretical Mendelian ratios noted. (See text p. 164 for explanation.)
[4] The *B* v_1 plants could not be satisfactorily classified, due to lack of vigor; hence, all v_1 plants were omitted in calculating the recombination percentage.
[5] Recombinations so rare that percentages were not calculated. Plants classed in recombination classes might be accounted for through contamination.

and absence of both *R* and *Y*. If this recessive female parental plant is partially self-fertile, an excess of recessives and a deficiency of *R* and *Y* plants is expected in the backcross population. Keller (*6*) has indicated some trouble with these partially self-fertile plants when used as female parents for backcrossing. In the present studies a small amount of selfing may have occurred in obtaining backcross populations, but precautions were taken to avoid self-fertile or partially self-fertile plants as female parents. If selfing occurred in backcrossing, the relative percentage was so low that it is hardly noticeable, and in general the observed ratios agree well with the theoretical 1 : 1 ratios (table 1).

Mendelian ratios were investigated for all populations listed in table 1, and discrepancies between theoretical and observed ratios were noted. The Mendelian ratios for linkage data between *B* and *Y* show a deficiency of *B* plants. This deficiency was expected because in this case environmental conditions were not optimum for classification of *B* plants. The percentage recombination (27.3) between *B* and *Y* (table 1), which appears to be too high, is, therefore, questioned. Ratios for linkage data between *B* and v_1 also show a deficiency of *B* plants, but this was in spite of optimum conditions for classification of annual types with a long period of growth under warm temperatures and continuous light (24-hour day). It is believed that the apparent deficiency of *B* plants was caused by the lack of chlorophyll in *B* v_1 plants, which interfered with growth and initiation of seedstalks. The *B*+ (or *B* V_1) plants, however, had excellent opportunity to bolt, so the recombination fraction was calculated from these classes, with variegated (v_1) plants not included.

Other departures from theoretical Mendelian ratios presented in table 1 may be noted as follows: A deficiency of v_1 plants in the distribution 75, 12, 24, 59 for *R* and v_1 linkage data; a deficiency of *Tr* and *Y* plants in the distribution 52, 274, 404, 54 for *Tr* and *Y* linkage data; and a deficiency of *r* plants in the F_2 distribution 315, 205, 137, 0 for *R* and *Tr* linkage data. It is not easy to explain some of these departures from the theoretical ratios. Such departures, however, do not interfere with conclusions regarding estimates of recombination percentages.

Average and combined values for the recombination percentages derived in table 1 are shown in skeleton form in table 2. In most cases these values are more accurate, because of more extensive data, than those derived from the 3- and 4-point tables (tables 3 and 4), but the information from the 3- and 4-point tables is helpful in establishing the order of genes.

TABLE 2.—*Summary of recombination percentages*

	Y	*R*	*Cl*	*Tr*	*Cv*	*B*	v_1
Y		8.4	11.4	12.7		[1] 27.3	24.6
R	8.4		3.8	4.3	4.3	15.9	17.7
Cl	11.4	3.8		0 ?			
Tr	12.7	4.3	0 ?		0 ?		
Cv		4.3		0 ?		12.2	16.5
B	[1] 27.3	15.9			12.2		10.0
v_1	24.6	17.7			16.5	10.0	

[1] The recombination percentage (27.3) between *Y* and *B* is questioned because of difficulty in classifying *B* plants, as explained in the text.

The arrangement of the data in the 3- and 4-point tables is similar to that employed by Emerson, Beadle, and Fraser (*4*) for maize. Under parental genotype the plus sign (+) refers to the normal allelomorph of the dominant or recessive gene designated by a literal symbol. The phenotypes for respective populations may be reestablished from the order in the following example:

F_1 genotype	Parental combinations		Recombinations					
			Region 1		Region 2		Regions 1 and 2	
$\frac{A\ B\ C}{+\ +\ +}$	(*A B C*) 162	(+ + +) 176	(*A* + +) 10	(+ *B C*) 7	(*A B* +) 6	(+ + *C*) 4	(*A* + *C*) 0	(+ *B* +) 3
Total	338		17		10		3	
Percent	91.9		4.6		2.7		0.8	

It can be noted in this example that there are two phenotypes for each classification, the one to the left beginning with a dominant allelomorph. The recombination percentages listed under region 1 are for single recombinations, and the total crossing over in region 1 is obtained by adding the single recombinations and the double recombinations. The recombination percentages for the three genes in the above example are *A B*, 5.4; *B C*, 3.5; and *A C*, 7.3.

Information presented in tables 2, 3, and 4 leads to the assumption of the sequence and approximate spacing of genes as follows:

Y 8 *R* 4 *Cl* (*Cl* 0 ? *Tr* 0 ? *Cv*) *Cv* 12 *B* 10 v_1

TABLE 3.—*Three-point linkage tests*

F_1 genotype	Parental combinations		Recombinations						Total	Recombination percentages
			Region 1		Region 2		Regions 1 and 2			
$\frac{Y\ R\ Cl}{+\ +\ +}$	162	176	10	7	6	4	0	3	368	
$\frac{Y\ +\ +}{+\ R\ Cl}$	440	456	45	49	12	16	0	0	1,018	
$\frac{Y\ R\ +}{+\ +\ Cl}$	179	177	6	14	13	12	1	0	402	
Total	1,590		131		63		4		1,788	*Y R*, 7.5 *R Cl*, 3.7 *Y Cl*, 10.8
Percent	88.9		7.3		3.5		0.2			
$\frac{Y\ R\ Tr}{+\ +\ +}$	29	43	5	6	1	1	0	0	85	
$\frac{Y\ +\ +}{+\ R\ Tr}$	689	655	70	62	20	39	2	0	1,537	
Total	1,416		143		61		2		1,622	*Y R*, 8.9 *R Tr*, 3.9 *Y Tr*, 12.6
Percent	87.3		8.8		3.8		0.1			
$\frac{Y\ +\ +}{+\ R\ B}$	157	129	19	23	23	44	3	2	400	
Total	129		19		23		2		[1] 173	*Y R*, 12.2 *R B*, 14.5 *Y B*, 24.3
Percent	74.6		11.0		13.3		1.2			
$\frac{Y\ +\ +}{+\ R\ v_1}$	153	121	11	12	25	42	10	5	379	
Total	153		12		42		10		[2] 217	*Y R*, 10.1 *R* v_1, 23.9 *Y* v_1, 24.8
Percent	70.5		5.5		19.4		4.6			

See footnotes at end of table.

TABLE 3.—*Three-point linkage tests*—Continued

F_1 genotype	Parental combinations		Recombinations						Total	Recombination percentages
			Region 1		Region 2		Regions 1 and 2			
$\frac{Y + +}{+ B\ v_1}$	169	34	9	44	33	17	7	94	407	
Total	169		44		17		7		[2] 237	$Y B$, 21.5 $B v_1$, 10.1 $Y v_1$, 25.8
Percent	71.3		18.6		7.2		2.9			
$\frac{R + B}{+ Cv +}$	680	714	33	36	112	86	1	5	1,667	
Total	1,394		69		198		6			$R Cv$, 4.5 $Cv B$, 12.3 $R B$, 16.0
Percent	83.6		4.1		11.9		0.4			

[1] Only bolters are considered since these were known to have the factor *B*, but all *B* plants did not bolt.
[2] The v_1 plants were not included because of interference with growth, due to lack of chlorophyll, which affected viability and the bolting rate.

TABLE 4.—*Four-point linkage test*

F_1 genotype	Parental combinations		Recombinations														Total	Recombination percentages
			Region 1		Region 2		Region 3		Regions 1 and 2		Regions 1 and 3		Regions 2 and 3		Regions 1, 2, 3			
$\frac{Y + + +}{+ R\ B\ v_1}$	164	31	3	16	6	28	24	17	5	3	6	0	1	94	9	0	407	$Y R$, 11.4 $R B$, 14.3 $B v_1$, 10.1 $Y B$, 21.5 $Y v_1$, 25.8 $R v_1$, 23.6
Total	164		16		28		17		5		6		1		0		[1] 237	
Percent	69.2		6.8		11.8		7.2		2.1		2.5		0.4		0.0			

[1] v_1 plants are not considered in calculating recombination percentages due to interference with expression of *B* factor.

Factors *Cl*, *Cv*, and *Tr* were found to be very closely linked, and their order has not been determined. Until further evidence is obtained the possibility of these three factors being allelic may be open to consideration.

DATA RELATING TO GENES NOT IN THE Y-R-B LINKAGE GROUP

Data for genes not in the *Y–R–B* linkage group are presented in tables 5 and 6. Table 5 has the same arrangement as table 1, except for the three columns to the right dealing with χ^2 values. The purpose of the χ^2_X and χ^2_Y values is to show whether or not differences between observed and theoretical Mendelian ratios have significance, and χ^2_L values were calculated for the purpose of revealing significant association or linkage between respective genes. The χ^2_X and χ^2_Y values were calculated in a similar manner from formulas given by Mather (7) as follows:

$$\chi^2_X = \frac{(X-x)^2}{n} \text{ for backcross data (where } X = a+b \text{ and } x = c+d)$$

and

$$\chi^2_X = \frac{(X-3x)^2}{3n} \text{ for } F_2 \text{ data.}$$

The χ^2_L values were calculated by the formulas

$$\chi^2_L = \frac{(ad-bc)^2 n}{(a+b)(c+d)(a+c)(b+d)}.$$

There is only one degree of freedom for each of these χ^2 values, and from Fisher's tables, as stated by Mather (7), it is seen that a value of

$\chi^2=3.841$ is required for the 5-percent point of significance and a value of $\chi^2=6.635$ is required for the 1-percent point of significance.

TABLE 5.—χ^2 *tests for linkages and deviations in Mendelian ratios for factors not in Y-R-B group*

Genes (*X Y*)	Linkage phase	Female parent	Families	Individuals					χ^2_X	χ^2_Y	χ^2_L
				$\frac{XY}{(a)}$	$\frac{Xy}{(b)}$	$\frac{xY}{(c)}$	$\frac{xy}{(d)}$	Total (*n*)			
			Number	*Number*	*Number*	*Number*	*Number*	*Number*			
bl pl	RF_2		3	186	49	61	20	316	0.07	1.69	0.53
bl ru	RF_2		1								
bl R	CB	Rec.	4	451	347	[1] 355	[1] 266	1,419	22.08	26.25	.06
	CB	Dom.	4	207	200	[1] 139	[1] 132	678	27.28	.29	.01
	CB (total)		8	658	547	494	398	2,097	46.72	20.43	.12
	CF_2		10	985	332	269	62	1,648	21.23	1.05	6.10
bl Y	CB	Rec.	1	13	12	16	16	57	.86	.02	.22
pl R	F_2		3	192	55	51	18	316	1.69	.61	.44
ru R	RB	Rec.	2	49	50	54	53	206	31	.00	.02
	RB	Dom.	1	91	112	90	81	374	2.74	.39	2.26
	RB (total)		3	140	162	144	134	580	.99	.25	1.71
	RF_2		1	102	22	29	14	167	.50	1.06	4.14
ru Y	CB	Rec.	1	51	48	49	58	206	.03	.17	.68
	CB	Dom.	1	109	94	82	89	374	2.74	.17	1.22
	CB (total)		2	160	142	131	147	580	.99	.01	1.99
	CF_2		1	90	34	38	7	169	.24	.05	2.53

[1] Difficulty was encountered in classifying some of the *bl* plants; approximately 10 percent of those classified as *bl* were somewhat questionable.

TABLE 6.—F_2 *data for ru and bl*

Date classified	Individuals						Total
	R			*r*			
	Normal root	Russet root	Black root	Normal root	Russet root	Black root	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Aug. 13, 1935	45	24	0	12	9	0	90
Sept. 14, 1938	78	46	9	20	11	1	165
Sept. 22, 1939	12	6	1	2	4	0	25
Sept. 24, 1940	123	52	2	26	14	1	218
June 15, 1940 [1]	182	82	30	68	24	5	391
Total	440	252		128	69		889
Ratio expected, 27:21:9:7	375.0	291.7		125.0	97.2		889

[1] Plants classified on June 15, 1940, were grown in the greenhouse, while those in other classifications were grown in the field.

Discrepancies in ratios for *bl* are seen (table 5) and were expected because in certain families difficulties in classification were encountered as well as a certain amount of mortality of *bl* plants. In some of the same families, however, discrepancies between observed and theoretical ratios for *R* are seen, and these discrepancies are not readily explainable.

The values for χ^2_L from F_2 data in table 5 reveal two cases of apparent deviation from independent assortment between factors. One

case (χ^2_L=6.10) is a deviation from independent assortment between *bl* and *R*, and the other case (χ^2_L=4.14) is a deviation from independent assortment between *ru* and *R*. The backcross data for these same combinations of factors, however, clearly indicate independence of inheritance.

A test for linkage between *bl* and *ru* and additional linkage tests between *bl* and *R* and *ru* and *R* are considered in table 6. A black-root plant homozygous for *bl* and *r* was crossed to a russet-root plant homozygous for *ru* and *R*. All F_1 plants had perfectly normal roots. The F_2 plants were grown in 4 different years. With independent inheritance, one-fourth of the total of 889 plants (table 6) were expected to show the black-root condition. Only 49 black-root plants were observed, showing a definite deficiency. There was considerable variation in degree of the russet-root condition, indicating that some *bl* plants were classified as russet rather than black root. The class designated as black root was very distinct in the field but not especially distinct in the greenhouse. Because of inability to separate all the *bl* plants from the *ru* plants the total black-root and russet-root classes are combined in table 6. After making this adjustment, the observed results agree fairly well with expected results, assuming independence between *R*, *bl*, and *ru* (table 6).

From the foregoing considerations independent inheritance is indicated between *bl* and *pl*, *bl* and *R*, *bl* and *Y*, *pl* and *R*, *ru* and *R*, and *ru* and *Y*. It is likely, therefore, that *bl*, *pl*, and *ru* are not in the *Y–R–B* linkage group. The evidence indicates that *bl* and *pl* are also in separate linkage groups. Data in table 6 indicate that *bl* and *ru* may be in separate linkage groups, although the linkage test for these two factors was not entirely satisfactory. The possibility of linkage between *pl* and *ru* was not tested. Thus, three linkage groups are represented: *Y–R–B*, *bl*, and *pl*. The factor *ru* may or may not represent a fourth group.

EFFECT OF RESPECTIVE GENES ON VIGOR AND PLANT WEIGHT

Factors *bl*, *Cl*, *Cv*, *pl*, *ru*, *Tr*, and v_1 all interfered more or less with vigor. The worst offender was v_1, and, as already stated, special precautions were necessary to secure survival of plants homozygous for v_1.

TABLE 7.—*Association of factors bl, ru, Cl, Cv, Tr, and Y with plant weight*

Factors compared	Number of plants		Average weight		Weight of mutant type as percent of normal
	Mutant type	+ (normal)	Mutant type	+ (normal)	
			Grams	*Grams*	
bl and +	618	1,521	6.1	62.1	10
ru and +	283	374	93.8	145.7	64
Cl and +	394	395	53.0	100.0	53
Cv and +	144	94	11.9	45.5	26
Tr and +	786	558	116.0	158.0	73
Y and +	227	198	209.6	142.8	147

Data for plant weights presented in table 7 refer to total plant weights (roots and tops). For each population reported the seedlings were allowed to grow without thinning. Care was taken to avoid

exposing to curly top the plants used in obtaining records of weights because of the correlation between at least one factor for curly top resistance and the color factor *R* (*2*).

The *bl*, *Cl*, and *Cv* plants were definitely lacking in vigor, while the *ru* and *Tr* plants also showed some decrease in vigor (table 7). The lack of vigor in *Tr* and *Cv* plants was less apparent in plants heterozygous for these factors than in homozygous *Tr Tr* or *Cv Cv* plants.

Plants carrying the *Y* factor have never appeared to be lacking in vigor, and limited data in table 7 indicate some increase in vigor with *Y* plants.

AN EXAMPLE OF CYTOPLASMIC INHERITANCE

A white variegation common to commercial varieties of sugar beets is illustrated in figure 7. This albino condition is believed to be similar to types described by Munerati (*8*). These partially albino plants are relatively rare, but when they do occur they are sometimes conspicuous. Some varieties seem to produce more variegated plants than others. The curly top resistant variety U. S. 22 produces occasional plants of this type.

In a 2-acre seed field grown to variety U. S. 22 in 1937, a variegated plant with partly white and partly green inflorescence was observed to be flowering and producing seed. Many plants of U. S. 22 that have been tested, all have been strongly self-sterile; hence, the flowers of this partially variegated plant were very probably fertilized by pollen from surrounding plants with normal chlorophyll. After ripening, the seed produced by the completely white or albino portion of the plant was collected and labeled "lot A." The seed produced by the portion of the plant that was chiefly green but had a few small white sectors was labeled "lot B." Observation of the seedlings grown from these two seed lots indicates maternal or cytoplasmic inheritance of material necessary

FIGURE 7.—Chlorophyll deficiency considered as an example of cytoplasmic inheritance in *Beta vulgaris*.

for the production of chlorophyll. Seedlings from lot A were all albino and died in the cotyledon stage, with 2 exceptions out of a total of 74 plants observed. Both albino and normal seedlings were produced by the seed labeled "lot B." These observations and the conclusions in regard to the cytoplasmic nature of inheritance are in agreement with observations and conclusions made by Munerati (*8*).

DISCUSSION

The expression of the Mendelian factors responsible for pigment development was markedly affected by environmental conditions, age, and stage of plant development. The red anthocyaninlike pigment produced in the presence of *R* is most conspicuous in hypocotyls of seedlings and in the crown buds of older vegetative plants. When the seedstalk is first initiated the anthocyaninlike pigment usually fades or disappears for a time, but it reappears as soon as vegetative or semivegetative buds develop on branches of the seedstalk. Injury, as from some insects and fungi, frequently causes the pigment to develop. The pigment produced under any of these conditions is some shade of red when the factor *R* is present, and some shade of yellow or orange in *r* plants.

Red pigment also develops in the pericarp of the seed ball on *R* plants. The red pericarp varies in intensity but was present in all strains tested. This was demonstrated by placing paper bags over the inflorescence and removing the bags when the seed balls were well formed just before ripening. A brilliant red color developed in the pericarp of *R* plants a few days after the bags were removed. This pigment gradually faded in the intense sunlight as the seed dried and ripened.

In general the red anthocyaninlike pigments and related yellow pigments in beets are increased during vegetative development by increased soil fertility, spacing, and light intensity. Low temperatures also have a decided effect in increasing pigment development. The relative effects of certain environmental factors, however, are dependent upon the genetic constitution of the plants. The expression of factors *Cl* and *Cv* is dependent upon sunlight, and the more intense the sunlight the greater the pigment development. The expression of the factor *Tr* is not affected in this manner, and *Tr* plants as well as *Y* plants develop pigment in total darkness.

It is of interest to note the chemical composition of the red pigment that is produced in large quantities in the beet root in the presence of *R* and *Y*. This pigment is known as betanin, and Pucher, Curtis, and Vickery (*13*) state that "Betanin has been classified as an anthocyanin, but differs from typical anthocyanins in that it contains nitrogen * * * it is a glucoside of a nitrogenous nucleus related to the anthocyanidins."

SUMMARY

Data for 10 Mendelian characters in *Beta vulgaris* L. are presented. These characters and their genetic symbols are as follows: *B*, bolting or annual habit; *bl*, black root; *Cl*, colored leaf; *Cv*, colored leaf veins; *pl*, plantainlike leaf venation; *R*, red plant color; *ru*, russet root; *Tr*, trout or spotted leaf; v_1, variegated foliage; *Y*, heavy production and extension of pigment.

Seven of the ten genes were found to be in the *Y–R–B* linkage group. The sequence and approximate genetic spacing of these genes were found to be as follows:

$$Y\ 8\ R\ 4\ Cl\ (Cl\ 0\ ?\ Tr\ 0\ ?\ Cv)\ Cv\ 12\ B\ 10\ v_1$$

The genes *Cl*, *Cv*, and *Tr* were very closely linked, and their order was not determined. According to available data, the genes *bl*, *pl*, and *ru* are not in the *Y–R–B* group.

All 10 genes, except *Y*, *R*, and *B*, interfered more or less with vigor. Limited data are presented showing the extent of loss in vigor associated with the presence of the genes *bl*, *Cl*, *Cv*, *ru*, and *Tr*.

A variegated foliage condition with large albino sectors was observed, and the type of progeny obtained from the albino and green sectors gave evidence of maternal or cytoplasmic inheritance.

LITERATURE CITED

(1) Abegg, F. A.
1936. A genetic factor for the annual habit in beets and linkage relationship. Jour. Agr. Res. 53: 493–511, illus.

(2) ———
1941. List of characters and gene symbols reported for the species Beta vulgaris L. Amer. Soc. Sugar Beet Tech. Proc. 1940: 109–113.

(3) Carsner, Eubanks, and others.
1933. Curly-top resistance in sugar beets and tests of the resistant variety U. S. No. 1. U. S. Dept. Agr. Tech. Bul. 360, 68 pp., illus.

(4) Emerson, R. A., Beadle, G. W., and Fraser, A. C.
1935. A summary of linkage studies in maize. N. Y. (Cornell) Agr. Expt. Sta. Mem. 180, 83 pp.

(5) Immer, F. R.
1930. Formulae and tables for calculating linkage intensities. Genetics 15: [81]–98, illus.

(6) Keller, Wesley.
1936. Inheritance of some major color types in beets. Jour. Agr. Res. 52: 27–38.

(7) Mather, K.
1938. The measurement of linkage in heredity. 132 pp., illus. London.

(8) Munerati, O.
1928. L'hérédité de l'albinisme en Beta vulgaris L. 5th Internatl. Kongr. f. Vererbungswiss. Verhandl. (1927) 2: [1137]–1142.

(9) ———
1931. L'eredità della tendenza alla annualità nella comune barbabietola coltivata. Ztschr. f. Zücht., Reihe A., Pflanzenzüchtung 17: [84]–89, illus.

(10) ——— and Costa, T.
1930. Osservazioni sulla trasmissione del carattere "pelle nera" nella barbabietola. Ztschr. f. Induktive Abstam. u. Vererbungslehre 54: [458]–465.

(11) Nuckols, S. B.
1931. Seedling color and yield of sugar beets. Amer. Soc. Agron. Jour. 23: 740–743.

(12) Owen, F. V., Carsner, Eubanks, and Stout, Myron.
1940. Photothermal induction of flowering in sugar beets. Jour. Agr. Res. 61: 101–124, illus.

(13) Pucher, George W., Curtis, Lawrence C., and Vickery, Hubert Bradford.
1938. The red pigment of the root of the beet (Beta vulgaris). I. The preparation of betanin. II. A method to determine betanin. Jour. Biol. Chem. 123: 61–75.

U. S. GOVERNMENT PRINTING OFFICE: 1942